STUDIES ON THE FORMATION AND FUNCTIONING OF SECONDARY PHLOEM IN SOME TROPICAL FRUIT TREES

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"In the name of thy Lord who createth,
Createth man from a clot.
And thy Lord is the Most Bounteous,
Who teacheth by the pen,
Teacheth man that which he know not."

- Alkoran

C E R T I F I C A T E

This is to certify that the dissertation entitled "Studies on the formation and functioning of secondary phloem in some tropical fruit trees," submitted to the Aligarh Muslim University, Aligarh, in partial fulfilment of the requirements for the award of the degree of Master of Philosophy is a faithful record of the bonafide research work carried out by Miss. Farzana Farooqui. No part of the dissertation has been published or submitted for any other degree or diploma.

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A C K N O W L E D G E M E N T

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C O N T E N T S

I N T R O D U C T I O N

Phloem is the principal food conducting tissue of the vascular plants. Like xylem, it is also a complex tissue and is composed of several kinds of cells concerned with different functions. It is generally made up of sieve-elements, parenchyma, fibres and sclereids. In some cases laticifers and various types of idioblasts, specialized morphologically and physiologically, also constitute this complex tissue system.

Phloem is a delicate tissue and possesses peculiar histochemical properties. The phloem cells other than the fibres and sclereids, do not develop such rigid, persisting walls as the xylem elements. The sieve-elements which are directly involved in the food-translocation, remain delicate, during their functional period. Later, when the phloem ceases to act as a conducting tissue, the sieve-elements become either generally disorganized or get greatly modified functionally and structurally. Thus in the phloem the conducting elements soon lose their original structure and appearance and therefore, it becomes necessary to investigate this tissue soon after its inception or before it ceases to become a non-conducting tissue.

Due to the lack of proper techniques and a strong stimulus from the commercial world, phloem as a tissue has not been studied to that extent as it has been done in the case of xylem. As a consequence, our knowledge for this tissue, still remains mostly incomplete, both regarding its structural variations and its phylogeny (Esau, 1965), although the discovery of phloem as

a conducting tissue, dates back to the first half of the previous century (Hartig, 1837).

Virtually nothing is known about the formation of secondary phloem and its duration of activity in the Indian tropical trees. Keeping this in view it was considered desirable to study the details of phloem development in some tropical fruit trees.

It is hoped that the information regarding the details of phloem development in the fruit trees may be of some help in controlling fruit production. Further, it may add to our knowledge about the structural variations of secondary phloem and its mode of development in a part of tropical flora which may be of prime importance in understanding the trends of phylogenetic specialization of this histologically and economically important tissue system in the tropical trees.

STRUCTURE OF PHLOEM

Phloem as a principal food conducting tissue forms a complex system, composed of various types of elements assigned with different functions. It develops as a peripheral vascular tissue and forms a part of the bark in the stems and roots of vascular plants. Depending on its origin it is resolved into primary and secondary phloem.

Primary Phloem :

The phloem differentiating in the primary plant body is termed as primary phloem. It is derived out of the procambium, the provascular tissue. It consists of protophloem elements (the first formed elements) and the metaphloem elements (the later formed elements). They vary in their development, structure and position.

Protophloem :

The portion of the phloem that differentiated first has been named 'protophloem' (Russow, 1872). Protophloem generally lacks well – defined sieve-plates and sieve-areas, and they are not associated with companion cells (Esau, 1965b; Fahn, 1967). However, in a recent communication Ghouse et al. (1972 , 1973) had reported the occurrence of well-developed sieve-areas on the lateral walls of the protophloem developed in the pine leaves.

Differentiation of protophloem generally precedes that of the protoxylem (Chauveaud, 1897, 1900; Griffiths and Malins, 1930; Chang, 1935; Esau, 1938a; Ghouse, *et al.* 1972) and takes place in acropetal order in angiosperms and gymnosperms (Reeve, 1942; Crafts, 1943a, b; Engard, 1944; Esau, 1943, 1945; Gunkel and Wetmore, 1946; Miller and Wetmore, 1946; Sterling, 1946). The sequence of proto-and metaphloem development was studied in detail by Schneider (1945) in peach and by Esau (1948) in the grapevine.

In angiosperms, sieve-tube elements had been observed in protophloem of roots, stems and leaves in woody and herbaceous species (Esau, 1939, 1950). These elements are narrow elongated with hardly distinct sieve-areas lacking companion cells. Their walls also show thickenings like that of sieve-elements.

The sieve-tubes of the protophloem function only for a brief period (Esau, 1965). By the enlargement of the surrounding cells, they are destroyed soon after maturation, since they are not able to keep pace with the growth of the organ. This is known as the obliteration of sieve-elements. The crushed cells may disappear completely later on.

In many dicotyledons the cells remaining after the obliteration of sieve-tubes transferred into fibres (Léger, 1897; Blyth, 1958). In leaves the remaining protophloem cells differentiated into elongated collenchymatically thickened unlignified cells. This type of transformation in leaves is widely distributed including in those which have got protophloem fibres (Esau, 1950).

These peculiarities of protophloem cells approached towards the erroneous assumption that it is distinct from the rest of the phloem and constitute a portion of the so-called pericycle (Blyth, 1958).

Metaphloem :

The part of primary phloem which arises after the protophloem, and matures after the growth of the surrounding tissues is completed, is termed as 'metaphloem' (Van Tieghem, 1887). In plants without secondary growth it froms the main conducting part of the plant after the development of primary body and persists for many years (Esau, 1965). In plants having secondary growth, this tissue becomes inactive after the formation of secondary elements, and either becomes crushed or obliterated completely (Esau, 1965).

The sieve-elements of metaphloem are longer and wider, with more prominent sieve-areas than the protophloem. In angiosperms these elements are sieve-tube members (Esau, 1965).

In dicotyledons, metaphloem contains companion cells and phloem parenchyma, while monocotyledons have sieve-tubes with their companion cells with no phloem parenchyma (Cheadle & Uhl, 1948). In such phloem, the sieve-elements and companion cells form a regular pattern and this feature is considered to be advanced phylogenetically (Carlquist, 1961).

According to Eames & Mac Daniels (1947), fibres occur in this tissue but Esau (1950) reports their absence from the

metaphloem of dicotyledons; if they are present in the primary phloem, they arise in the protophloem. Sometimes older metaphloem elements may become sclerified as in some herbaceous species (Esau, 1965).

The demarcation between proto- and metaphloem is clear in some cases as in aerial parts of monocotyledons due to the presence of only sieve-tubes in protophloem and sieve-tube members with companion cells in the metaphloem (Esau, 1965). In plants having secondary growth the demarcation between secondary tissue and metaphloem is difficult, except with the radial arrangement of cells in secondary phloem (Esau, 1965).

Secondary phloem :

The secondary phloem develops out of the outer derivatives of the vascular cambium. It is generally made up of sieve-tube members, companion cells, parenchyma, fibres and rays. In some cases sclereids also form a part of this tissue.

The sieve-elements of secondary phloem are usually shorter in size than the primary ones. They possess well developed sieve-areas and sieve-plates, and are arranged in radial files by which it is distinguished from the primary part of the phloem.

The outer derivatives of the cambium undergo periclinal (additive) divisions before they differentiate into phloem proper. But in conifers (Esau, 1965) the fusiform derivative

differentiates directly into a sieve-cell, usually, without being subdivided into smaller cells. In dicotyledons, the longitudinal divisions separate the future companion cells from the associated sieve-tube members. But the fusiform initial may divide by transverse, oblique or longitudinal divisions resulting in the formation of more than one sieve-element with their companion cells or sieve-elements, companion cells and parenchyma.

After these divisions get completed, sieve-tube members begin to differentiate by their complex cytologic changes, characteristic of them, and their primary pit-fields become modified into sieve-areas.

The fusiform initials, that form the axial parenchyma, usually divide by transverse or oblique divisions into smaller cells. Fibres differentiate from fusiform cells by developing secondary walls and with or without apical intrusive growth (Esau, 1965).

The phloem cells expand transversely to varied degrees as they diverge from the cambium. The sieve-tube members increase more in diameter than the fibres and the ray cells commonly change little. In some plants the ray and axial parenchyma cells differentiate into sclereids (Esau, 1965).

The phloem is considered to be differentiated into a conducting tissue when the sieve-elements become enucleate and develop the sieve-area strands between the cells.

Basic Structure :

The secondary phloem is composed of two main systems, the axial or vertical system, derived from the fusiform initials and the transverse or ray system derived from the ray initials. The sieve-elements (sieve-cells & sieve-tube members with their companion cells), phloem parenchyma and phloem fibres constitute the axial system while the principal component of the transverse system is the ray parenchyma cells.

Holdheide (1951) has reported in many woody dicotyledons, the division of secondary phloem into seasonal growth increments, although this is less prominent than in the secondary xylem. Many gymnosperms and angiosperms form fibres in tangential bands in the secondary phloem, but the number of bands cannot help in estimation of the age of the phloem tissue as this is not constant from season to season.

The phloem rays are continuous with the xylem rays and they together constitute the vascular rays. The xylem and phloem rays are same in height and width near the cambium as they arise from a common group of ray initials. However, the older part of the phloem ray which is displaced by the expansion of the secondary body may vary in its width (Holdheide, 1951). Before their dilation the phloem rays are similar to those of xylem rays in their form and size, in the same species. Phloem rays are uni, bi-or multiseriate. They vary in height; small and large rays may be present in the same species.

Rays may be heterogenous i.e. composed of 2 kinds of cells - the procumbent and erect - or they may be homogenous i.e. composed of only one kind of cell. Phloem rays do not attain the same height as xylem rays since the xylem production is larger than the phloem production by vascular cambium and also due to the fact that the outer portion of the phloem is peeled off by the activity of phellogen.

The axial system in dicotyledons constitutes the sieve-tube members with their companion cells, axial parenchyma and fibres, while those of the ray system are ray parenchyma cells. Both systems may contain sclereids, secretory elements, laticifers and various idioblasts with specialized contents. Crystal accumulation is common and occurs in phloem parenchyma and ray cells.

Storied, nonstoried and intermediate arrangements are found with uni-, bi- and multiseriate rays.

Distribution of fibres in the secondary phloem is different in different species (Möller, 1882; Strasburger, 1891; Holdheide, 1951; Zahur, 1959). They may occur in tangential bands, may be scattered or even absent in some cases.

In some genera as in those of Pomoideae (Evert, 1960, 1963a) the sieve-elements very much approach towards the sieve-cells of conifers (long elements with much inclined walls) having less distinction between the sieve-areas of the sieve-plates and those of the lateral walls. Certain woody dicotyledons

have non-stratified phloem with very long sieve-tube members bearing usually compound sieve-plates on the inclined end walls (Betula, Quercus, Populus, Tilia, Liriodendron, Aesculus, Juglans, Esau, 1965). In many structurally advanced dicotyledons the phloem is storied and possesses short sieve-tube members with slightly inclined (Fagus, Acer) or transverse end walls (Robinia, Ulmus) with simple sieve-plates.

The secondary phloem of herbaceous dicotyledons resemble that of woody species. However in some herbaceous species the secondary phloem resembles that of primary one (cucurbits). The secondary phloem in cucurbits consists of wide sieve-tubes, narrow companion cells and somewhat smaller phloem parenchyma cell. No fibres and rays are present. The sieve-plates are simple and lateral sieve-areas are less specialized than the sieve-areas of the simple sieve-plates.

In conifers the axial system of the secondary phloem consists of sieve-cells, parenchyma and frequently fibres. Rays are uniseriate, contain parenchyma with albuminous cells (Esau, 1965). The cell arrangement is non-storied, hence the radial seriation of cells is retained in the mature tissue due to the fact that the expansion of cells at the time of differentiation is uniform and there is a slight apical elongation. In general the conifer phloem shows little developmental disturbances in the arrangement of cells.

The sieve-cells are slender, elongated elements, overlapping each other at their ends and each is in contact with several rays. The sieve-areas are found on these overlapping end walls. However, the sieve-areas except on the end walls, are restricted to the radial walls (Strasburger, 1891; Abbe & Crafts, 1939).

The phloem parenchyma cells store starch at certain times of the year and contain resins and tannins also. Crystals are also deposited in phloem parenchyma.

Conducting Phloem :

The active part of the phloem is known as conducting phloem, the amount of which varies in different species as the wood, depending on the age of the tree and its growing conditions. The width of the conducting phloem, usually measures in fractions of a millimeter.

Although the active phloem constitues only a small portion of the bark, it is of utmost importance for the detailed structure of the bark, as all the characters such as shape and length of sieve-element; presence of nacreous walls in sieve-element; structure of the sieve-plates; companion cells; variation of parenchyma cells and the occurrence of divisions in phloem mother cells that reduce the potential size of the sieve-elements, are clearly visible in this part of the bark (Esau, 1964).

Non-conducting Phloem :

That part of the secondary phloem in which the sieve-tubes no longer serve as a conducting system has been termed as nonconducting phloem (Esau, 1965). The former term nonfunctioning phloem (Esau, 1950) is erroneous since the phloem in which the sieve-elements cease to function retains phloem parenchyma usually which continue to store starch and tannins until the tissue is damaged by the activity of phellogen.

The main characteristic features of the nonconducting phloem are the presence of definitive callose on the sieve-areas, the disorganization and disintegration of the protoplast and the crushing of the elements in the older regions of the phloem. The companion cells, some of the phloem parenchyma cells and in conifers the albuminous cells also cease to function and collapse. The nonconducting phloem hence composed of the crushed elements.

The characteristics of the inactive phloem as a whole vary in different plants. In some dicotyledons, like Liriodendron (Cheadle & Esau, 1964), Tilia, Populus and Juglans there is a slight change in the shape of the functionless sieve-tubes. In others like, Aristolochia and Robinia, the sieve-elements and their associated cells collapse completely. In conifers the collapse of the old sieve-cells is very marked (Abbe & Crafts, 1939). In Vitis vinifera the nonconducting sieve-tubes become filled with tylose like structures arising from their neighbouring axial parenchyma cells (Esau, 1948).

The non-conducting phloem undergoes sclerification by the development of fibres or sclerieds from axial and ray parenchyma cells.

The amount of the non-conducting phloem depends on the manner of formation of phellogen. If the phellogen is superficial, and is not replaced by deeply-lying phellogens for many years, the plant may have the broad zone of non-conducting phloem (Prunus, Schneider, 1945). If on the contrary, the phellogen is formed year after year in deeper layers, the non-conducting phloem is in small amount (Vitis, Esau, 1948).

Periderm and Rhytidome :

Periderm formation is a universal feature in dicotyledons and conifers with the exception of few forms (Esau, 1965, p. 339). It arises in cortex either in the outer layers or in the deeper ones and isolate blocks of tissue (cortex and phloem) from the underlying tissues. This isolated part of the bark is known as the rhytidome (Esau, 1964), the crust of the bark.

REVIEW OF LITERATURE

History of Phloem :

The history of phloem as a principal conducting tissue goes back to the ninteenth century. Earlier to this only the fibres attracted the attention and the tissue as a whole was known by the name bast. It was only after the discovery of the sieve-tube elements by Hartig (1837), that the true nature of the tissue was revealed. Hartig found some elongated elements with sieve like structures on their walls and named them as the 'sieve-tubes'. He also recognized the complex nature of this tissue. Later the importance of sieve-tubes and parenchyma as the essential elements of the phloem was stressed by Von Mohl (1855). The term 'phloem' for the entire tissue, was proposed by Nägeli (1858) leaving the term 'bast' for the fibres only.

The companion cells were recognized and named by Wilhelm in 1880 as specialized parenchyma cells ontogenetically related to the sieve-elements.

Structure and development of sieve-elements :

Sieve-elements as conducting bodies were first discovered by Hartig in 1837 and later they were recognized by subsequent workers as the principal conducting elements of the phloem. Hartig (1837) who discovered the sieve-tube, had given indication that he was aware of the difference between sieve-cells and sieve-tubes as he only applied the term sieve-tubes to the tubular structures subdivided into their individual units by somewhat inclined terminal walls. The conducting nature of sieve-elements was further made clear when Cheadle and Whitford (1941) demonstrated that the elements bear pit like structures where pores with connecting strands are grouped together constituting the sieve-areas. They further distinguished that part of the wall which has highly specialized sieve-areas with more conspicuous connecting strands as sieve-plate.

Cheadle and Whitford (1941) called the conducting elements of the phloem as 'sieve-elements', but they separated the less specialized elements having sieve-areas on all walls as 'sieve-cell from the 'sieve-tube members' which are characterised with highly specialized sieve-areas grouped together to form the sieve-plate. It is interesting to note in this connection that the two types of sieve-elements i.e. sieve-cells and sieve-tube members differ in the degree of differentiation of their sieve-areas and their distribution on the walls. A sieve-cell is an element having unspecialized sieve-areas with no marked difference between each other and bearing no sieve-plate like structure on their

end walls. They are long, slender with tapering or somewhat steeply inclined end walls. In the tissue they overlap each other.

Sieve-tube members are the elements having some highly specialized sieve-areas localized on their end walls in the form of sieve-plates. Their end walls vary from much inclined to transverse. These units are placed end to end, their common walls bear the sieve-plates, thus forming a continuous tubular structure.

Sieve-areas :

The peculiarity in the sieve-elements is due to their specialized wall areas i.e. the sieve-areas. Hartig who introduced the term sieve, supposed that the walls of sieve-tubes in Cucurbita are perforated in a manner of sieve (Hartig, 1854). Von Mohl (1855) saw a thin membrane obstructing the pore. Nägeli (1861, 1863) also agreed with the name sieve-tube given by Hartig, since their terminal walls were perforated.

Sieve-areas are wall areas, comparable to primary pit-fields, with pores through which they are connected to the adjoining sieve-member by means of cytoplasmic strands. The term sieve-area was proposed by Cheadle and Whitford (1941). Theyare actually specialized primary pit-fields in which the diameter of the pores ranges from a fraction of a micron to 15 and may be more in some dicotyledons (Esau and Cheadle, 1959).

Development of the pores in sieve-area :

The development of pores in the sieve-area was studied in Vitis by Hill (1908) and later in Acer pseudoplatanus by Northcote and Wooding (1966), in Cucurbita maxima by Esau and Cheadle (1965) and Evert et al. (1966) , in Nicotiana tobacum by Wooding (1969) and Anderson and Cronshaw (1970). Hill (1908) described the development of pores in the following manner.

The pores of developing sieve-areas or the primary pit-fields are traversed by plasmodesmata. As the cell-wall of the sieve-element increases in thickness the plasmodesmata become longer and more prominent. At this time, small amounts of callose appear at the ends of the thread. With further increase in wall thickness the callose appreaches towards the middle lamella forming a 'coating' around each thread. He concluded that the 'rods' or cylinders of callose do not touch each other at the middle lamella, resulting in the formation of a median nodule or small cavity separating the two opposed cylinders of callose. The callose-lined canals then widen and perforation of the pore is completed. He assumed that the plasmodesmata of the young sieve-areas are attacked by enzymes (which he named 'ferments') which bore out the holes in the wall. He further suggested that the median nodule arose through the action of enzymes on a portion of middle lamella between callose cylinders.

The development of the sieve-plate was studied by Jarczewski (1878) and Wilhelm (1880). The latter considered the inclined

pitted end walls of young sieve-tubes of Vitis, represented as the future cell-plate. According to him, Cucurbita also shows the same sieve-plate ontogeny as Vitis except that only one sieve-plate develops on each end wall.

Sieve-plate :

The structure of the sieve-plate is quite complicated. It consists of two primary walls containing cellulose and polyuronides, connected together by an intercellular substances (middle lamella) of polyuronides (Abbe and Crafts, 1939).

Sieve-plates in sieve-tubes of different regions of the phloem and of different plants vary with regard to the kind, number and arrangement. A transverse end wall usually bears only one sieve-plate while an inclined wall has more than one sieve-plates.

Nägeli (1861) found small sieve-plate like structures on the longitudinal walls of sieve-tubes and referred them as the 'sieve-fields'. According to Hill (1908), sieve-plates and sieve-fields are the same structures. These sieve-fields connect the sieve-tubes not only with other sieve-tubes but also with certain parenchymatous members of the phloem (Hill, 1908). In gymnospermous sieve-elements and albuminous cells, similar connections were observed (Strasburger gave the name 'one-sided sieve-pits' to this type of wall structure). Strasburger (1901) compared the sieve-fields of gymnosperms and angiosperms and found that in the former, the closing membrane of the sieve-field remains intact while in the latter, it dissolves, so that the

sieve-field reduces to a single strand and a single pore. Haberlandt (1914) and Eames and MacDaniels (1947) gave the same interpretation about the sieve-fields of angiosperms and gymnosperms. Strasburger (1891, 1901) employed the term 'sieve-pits' (Siebtüpfel) to the less developed sieve-plate like structures on the lateral walls. Several workers (Hemenway, 1913; Jeffrey, 1917; MacDaniels, 1918) gave the name 'lattice' to the Nägeli's 'sieve-fields'. However, others (Schmidt, 1917; Crafts, 1932, 1939) called the 'sieve-pits, simply, 'pits'.

Callose :

The protoplasmic strands are commonly associated with a carbohydrate 'callose', a polymer of glucose residues united into spirally wound chains in β-1-3 linkages (Kessler, 1958).

Hartig (1837) who reported the pores on the wall, also recognized the complexity of the structure of the sieve-plate in Cucurbita. He observed the callose, lining the pore but did not name it. Nägeli (1861) referred it as 'soft substance' and in Cucurbita reported it as collars of varied thickness around the connecting strands.

Wilhelm (1880) observed that anilin blue is absorbed by the callose and Zinc Chloride and Potassium Iodide stain it intense red. Russow (1881, 1882a) demonstrated its presence in 220 spp. of 75 families, including angiosperms, gymnosperms and cryptogams.

The term 'callose' (Mangin, 1892) was derived from 'callus', a word first employed by Hanstein (1864) in connection with the study of old sieve-elements having a massive accumulation of callose on their sieve-areas. Mangin in 1892 found that there is a substance present in some plant cells which stains like the sieve-tube callose, but its chemical nature was not known to others like Schmidt (1917) and Rawlins(1933).

Wilhelm (1880) reported in some cases that large masses of callose represent a temporary cessation of activity e.g. in Vitis, Cucurbita and Aristolochia. In Vitis he found that this provisional callose dissolves out in spring and at the end of the second season, the same sieve-tube again forms the callose and ceases to function. Janczewski (1881) also reported provisional callose in winter in Tecoma and some monocotyledons. However, Janczewski (1881), Strasburger (1891) and others gave the idea that callose accumulation depends upon the age of the sieve-tubes and not on the season, in majority of the plants.

The deposition of callose takes place in two manners. In 1889, Leconte, first proposed the term 'cal definity' (definitive callose) to that type of deposition which is associated with a complete cessation of function of the sieve-element and the callose accumulation which occurs during a temporary break in activity as in Vitis, is termed as 'Dormancy or Provisional callose' (Esau, 1939, 1948).

Callose is deposited rapidly on walls in response to injury (Eschrich, 1956; Currier, 1957). In mature and conducting sieve-elements, the amount of callose is relatively small.

However, Eschrich (1963) has raised the question whether callose is present in conducting sieve-elements in plants or not, but the answer was already given by the former work of Esau, Cheadle and Gifford Jr. (1953) who demonstrated that the deposition and distribution of callose is so characteristic that it may be successfully used as the diagnostic feature of the conducting cells of the phloem.

Recent work regarding the deposition of the callose on sieve-areas also supports the view that it is deposited in response to injury during manipulation and fixation of the tissue (Evert and Derr, 1964; Northcote and Wooding, 1966; Currier and Shih, 1968; Wooding, 1968; Behnke, 1969; Cronshaw & Anderson, 1969).

Several factors like changes in pH or osmotic values, mechanical irritants, chemical stimulation, daylength etc. had been found concerned with the formation or removal of callose (Eschrich, 1957, 1961, 1963; Crafts and Currier, 1963; Ullrich, 1963). Eschrich (1961) had reported that out of these, pH values play an important role.

Many suggestions had been given to explain the role of callose in plant cells. The most popular view was that it serves as a regulatory mechanism in the flow of substances through the sieve-areas by narrowing the connecting strands and it also serves as a protection against the leakage of sap constituents into the walls at the sieve-areas (Currier and Strugger, 1956; Currier, 1957; Esau and Cheadle, 1959; Crafts and Currier, 1963).

Evert, Murmanis and Sachs (1966) reported that callose is generally absent in the normal functioning sieve-elements of Cucurbita. On the other hand, Parthasarathy (1968) correlated its presence with the metabolic state of the sieve-element.

In monocotyledons also, callsoe was reported to occur on the sieve-areas and sieve-plates (Behnke, 1965; Lawton, 1966; Ervin & Evert, 1967, 1970; Parthasarathy, 1968).

Origin of callose :

There are different opinions of workers regarding the origin of callose. Wilhelm (1880), Janczewski (1881) and Oliver (1887) regarded it as a transformed cellulose of the cell plate while others (Hanstein, 1864; Russow, 1882a; DeBary, 1884; Strasburger, 1891; Schmidt, 1917) considered it as a deposit derived from the cytoplasm. On the other hand Fischer (1886) and Hill (1901) gave a relation between slime and starch of sieve-tubes to the callose. Lecomte (1889), Hill (1908) Sykes (1908) and Thoday (1911) considered that the callose was formed by alteration of cellulose but the large masses were derived from the cytoplasm. Contrary to these views, Salmon (1946) gave an implausible assumption that the callose is derived from the sieve-tube slime.

Wall structure :

Many workers like Lesage (1891) and Léger (1895; 1897a,b) studied the wall of the sieve-tubes of primary phloem in many species of all plant groups and observed thick glistening walls

having a pearly lustre. They referred them as 'nacré'. Although Lesage (1891) was not sure about the nature of the cells having this type of wall structure but Léger (1897b), however, concluded that they are sieve-tubes. Chauveaud (1897, 1900, 1911) found that these thickenings develop on the wall of the sieve-tubes at the time of development of sieve-plates in it. He also discarded the view (in 1911) that only sieve-tube elements have got this type of wall thickenings. The thick walls of sieve-tubes are peculiar for them only (Esau, 1938). According to Léger (1897b), Schmidt (1917), Zimmermann(1922) and others the wall is thinner at the corners of the sieve-tubes.

The walls showed high chromaticity with stains like Bismark Brown (Chauveaud, 1900) or Light Green (Chang, 1935). Zimmermann (1922) used a combination of Hematoxylin and Congo Red, that stained the outer wall voilet and the special inner one, red. These nacré walls swell readily with appropriate treatments (Schmidt, 1917) and are highly hydrated (Esau, 1936, 1938a).

Regarding the nature of the thick walls they are cellulosic (Schmidt, 1917). Further, Léger (1897b), Schmidt (1917) and Zimmermann (1922) described this wall as a layer laid over the primary wall but they could not confirm that nacré is a secondary wall. Reports of Janczewski (1881), Russow (1882a) and Hill (1901) had shown that the thick walls of pine sieve-elements are cellulosic and become thin as the sieve-element ages.

In contrary to these reports Sykes (1908) described the nature of these walls as mucilaginous and suggested their

occurrence in both the sieve-tubes and parenchyma cells. He also reported that they are easily affected by swelling agents and stain with methylene blue in contrast to the sieve-plates and pit-closing membranes. But Schneider (1945) confirmed the cellulosic nature of nacré walls. Crafts (1943) and Sterling (1946) working on gymnosperms found it to be doubly refractive.

Appearance of characteristic nacré wall thickening is not a permanant feature. Many workers (Fischer, 1885; Léger, 1895, 1897a, 1897b; Chauveaud, 1897, 1900; Chang, 1935; Esau, 1936, 1938a) had reported that the wall becomes thin like the parenchyma cell-wall, as the sieve-tube ages. Léger (1897b) reported that nacré wall is the characteristics in older organs of the plant than in the younger ones. Janczewski (1881) and Russow (1882a) described thick walls in newly differentiated secondary sieve-tubes in Pinus sylvestris. Hill (1901) later on considered these walls to be similer to the nacré walls of Léger. Esau (1939) also proved that this wall loses its thickening as the sieve-element ages, due to the loss of water. But this reduction in thickness is not a constant feature as Sterling (1946) had reported in case of Sequoia where the nacré thickening of protophloem cells persists until the cells were obliterated.

Some plants lack this thickening (e.g. Vitis) as reported by Esau (1948). Lesage, Léger and Chauveaud found nacré walls in phanarogams as well as in cryptogams. Russow (1872) also reported it in Marselia. It is also a characteristic feature of the sieve-tubes of Laminariaceae (Will, 1884; Perrot, 1899; Sykes,

1908). Will (1884) and Sykes (1908) also agreed with its cellulosic nature and they also observed striations on these walls.

Recent reports (Esau, 1965) confirmed the fact that the walls of sieve-elements are made up of cellulose. No well authentic report for their lignification is available as yet. Walls of sieve-elements vary in thickness (Esau, 1965). Esau and Cheadle (1959) also reported nacreous thickening in some species which gives a positive reaction to tests for cellulose and pectin. The wall is not hydrated exceptionally but it shrinks with the age of the sieve-element (Esau, 1965). It may be so thick as to reduce the lumen very much but it does not cover the sieve-areas. The wall in absence of nacreous thickening is regarded as primary by the light microscopic studies (Esau, 1965).

P-Protien (Slime body):

P-protien or the slime body is one of the characteristic structures of the active sieve-elements of dicotyledons and conifers. Wilhelm (1880) was the first to report these structures in Vitis and Cucurbita and to term them as 'slime-drops'. Russow (1882a) recorded the slime bodies only in dicotyledons and not in monocotyledons and pteridophytes which contained watery sieve-tube contents. Strasburger (1891), Baccarini (1892), Staritz (1893) and Mrazek (1910) studied the slime formation in Leguminosae. The former referred these structures as slime-bodies and found arising within the parietal cytoplasm and remaining intact until the sieve-tubes are obliterated.

Esau (1938a) also studied the slime bodies in Leguminosae and Solanaceae and found them as elongated bodies which become fibrous as they increase in size. Crafts (1932, 1933, 1934, 1939) on the other hand found the slime of matured sieve-elements as amorphous colloidal suspension. According to him the slime bodies attain a fibrous appearance when they enlarge and give rise to thread like strands after their dispersal.

Engleman (1963) described the slime as having fibriller structure but showing an amorphous granular nature in the fixed material. Engleman again in 1965 found amorphous or fibiller or both the types of slime structures in Impatiens. Evert and Derr (1964) obtained amorphous, fibriller, particulate or reticulate nature of slime.

On the other hand Shah & Thulasy (1969) got four types of slime structures in three species of cucurbits (amorphous slime, slime plug, slime strands and a mixture of strands and amorphous slime). According to these workers the type of slime one would get depends on the nature of fixative used. They obtained more number of slime-plugs when the materials were fixed in F.A.A., in the formalin fixed material the fourth type of slime structure was found to be rare and in Craff III fixed ones the slime plugs became more prominent than others.

This fibriller material, proteinaceous in nature had been recognised as an important constituent of matured sieve-elements in many dicotyledons (Esau, 1969), monocotyledons (Behnke, 1969) and in gymnosperms (Kollmann and Schumacher, 1963; Evert and

Alfieri, 1965; Shah and Thulasy, 1969). Recently the analysis of phloem exudate by physiochemical methods had proved the nature of the fibriller material to be only protein which may be of the structural rather than the enzymatic type (Kollmann et al., 1970; Kleinig et al., 1971; Weber and Kleinig, 1971; Walker and Thaine, 1971; Eschrich et al., 1971). Further Yapa and Spanner (1972) confirmed the proteinaceous nature of fibrils during their electron microscopic observations on Tetragonia and Lycopersicum.

Thaine (1962) reported transcellular strands in the sieve-elements and also observed particle movement along these strands. Thaine (1961, 1962) and Canny (1962) evolved a new translocation hypothesis on the general occurrence of these strands. Studies of Evert & Derr (1964) also indicated that slime is not only dispersed throughout the vacuole of the sieve-element but also occurs in the form of strands traversing the cell and running from cell to cell through the pores. They considered these strands to be made up of slime, homologous to the fine threads which were considered strand inclusions by Thaine (1961).

Therefore during the past several years considerable controversies have existed regarding the nature of the internal strands found in matured sieve-elements. Some believing in the view that they are membrane bound cytoplasmic strands (Thaine, Probine & Dyer, 1967), others support the concept of the presence of internal strands which are derived from the slime bodies of matured sieve-elements rather than cytoplasm (Evert & Derr, 1964; Evert & Murmanis, 1965; Evert, Murmanis & Sachs, 1966; Tamulevich

& Evert, 1966; Shah and Thulasy, 1969). However Cronshaw & Esau (1968) suggested that the strand like condition of slime or P-protein is an artifact which appears due to injury.

Ultrastructural studies under-taken in the recent years contributed much to our understanding of the nature of the P-protein components. Several workers are in favour of the view that P-protein occurs in two main forms, the tubular and the fibriller one (La Fleche, 1966; Northcote & Wooding, 1966; Tamulevich and Evert, 1966; Cronshaw & Esau, 1967; Esau et al., 1967; Wooding, 1967, 1969; Steer & Newcomb, 1969). While the workers including Northcote & Wooding (1966), Cronshaw & Esau (1967), Wooding (1967), Steer & Newcomb (1969) suggested that one form of P-protein, the wider tubular component, gives rise ontogenetically to the other form, the narrow, striated, fibriller one. This phenomenon had been observed in many plants like Acer pseudoplatanus (Wooding, 1967), Nicotiana (Cronsaw & Esau, 1967), Coleus bluma (Steer and Newcomb, 1969).

Ultrastructural investigations by Evert & Murmanis (1965), Evert et al. (1966) have revealed that even the finer strands observed with light and phase microscopy are aggregates of smaller units.

Recently Evert, Eschrich & Eichorn (1973) found no evidence for the presence of either transcellular strands or of axially-oriented microtubular structures in matured sieve-elements, as recorded earlier (Thaine, 1969; Fensom 1972). They also did not find any evidence for the 'even' distribution of P-protein

throughout the matured sieve-elements as reported by Wooding (1969) and Cronshaw & Anderson (1971).

With the available informations it is difficult to visualize the role of P-protein in the sieve-elements. Some believed (Thaine, 1961, 1962; Canny, 1962) that it plays a positive role in the translocation mechanism while the majority of workers did not subscribe to this view, since it involves at least two types of mechanisms of translocation one involving the plants having P-protein and the other in those which lack it. This appears to be highly improbable considering the general structure of sieve-element contents as a whole. Moreover the recent studies of Evert, Murmanis & Sachs (1966) revealed that the slime bodies in Cucurbita are bound by a double membrane. This feature, if it is present as a constant one, this will mean that the slime body is an organelle which is concerned with the synthesis of slime.

The numerous strands which run from one sieve-plate to the other in angiospermous sieve-elements are connected with the strands arising from the lateral sieve-areas. So the strands together compose a stationary system most of which are oriented with the direction of flow of assimilates. However the presence of slime or strands in phloem exudate as shown by Eschrich (1963) & Thaine (1964a, b) should not be considered as an evidence that the strands flow with the assimilate stream.

In addition to the extranuclear P-protein, nuclear P-protein has also been reported in many plants.

However, P-protein has been found lacking from a wide variety of vascular plants viz., Polypodium vulgare (Maxe, 1966), Metasequoia glyptostroboides (Kollmann & Schumacher, 1964), Welwitschia mirabilis (Evert et al., 1972a, b), Hordeum vulgare (Evert et al., 1971) and Zea mays (Singh & Srivastava, 1972). It is also known to be absent from some lower vascular plants other than Polypodium.

Protoplast :

The unusual characteristics of the sieve-element is its extremely unstable protoplast. Certain changes have been observed during differentiation of sieve-element and in connection with its seasonal activity.

Like the procambial cells from which they develop, young sieve-elements with a conspicuous nucleus have plasmalemma attached to the wall and a distinct tonoplast delimiting the large central vacuole. Kollmann (1964) observed in Metasequoia glyptostroboides that as the differentiation of the sieve-element progresses, cytoplasm undergoes some general changes simultaneously with the start of the nuclear disintegration. Following this the delimitation of the parietal cytoplasm from the vacuolar membrane (tonoplast) disappears and the protoplast has a distinct membrane only next to the cell wall (Hohl, 1960; Ziegler, 1960; Duloy, Mercer & Rathegeber, 1961; Esau & Cheadle, 1961, 1962a; Esau, Cheadle & Risley, 1962; Mehta and Spanner, 1962; Esau, 1963; Eschrich, 1963; Falk, 1964).

However in Passiflora (Schumacher & Kollmann, 1959; Kollmann, 1960) and in Metasequoia (Kollmann, 1961 ., ; Kollmann & Schumacher, 1961, 1962), occasional existence of tonoplasts in sieve-elements and in sieve-cells had been observed. In both the above plants there occurs the disappearance of tonoplast (Kollmann & Schumacher, 1964) during the differentiation. After the disappearance of tonoplast most of the cytoplasmic organelles still lie in the parietal protoplast (Hohl, 1960; Ziegler, 1960; Duloy et al., 1961; Esau & Cheadle, 1961, 1962a; Esau, Cheadle and Risley, 1962; Esau, 1963).

Velten (1872) was the first to observe the cytoplasmic streaming in the phloem. In Vicia napaea he observed this phenomenon in matured sieve-tubes while in Arundo donax in young sieve-tubes. Strasburger (1891) observed it in young sieve-tubes containing nuclei of Zea and Cucurbita. Huber (1932), Crafts (1932, 1933, 1934) and Abbe & Crafts (1939) found the lack of streaming in matured sieve-tubes.

Cytoplasmic Inclusions:

The nature of the various inclusions was discussed by Newcomer (1951), Millard and Bonner (1953), Goddard and Stafford (1954), Sorokin (1955a, b), and Hackett (1955).

In onion epidermal cells Perner (1952, 1953) and Drawert (1952, 1953) distinguished three types of granular bodies — mitochondria, plastids and spherosomes.

Sorokin (1955a) distinguished three types of mitochondria (the spherical, granular or cylindrical) in addition to the plastids and spherosomes.

Electron microscopic studies revealed a number of interesting structures. Several workers observed many well differentiated dictyosomes in earlier stages of development of the sieve-elements which have got a delimiting tonoplast (Schumacher and Kollmann, 1959; Kollmann, 1961a, b; Esau and Cheadle, 1962a, b; Esau, Cheadle and Risley, 1962; Kollmann and Schumacher, 1962b; Esau, 1963). During differentiation these dictyosomes disintegrate and gradually completely disappear in matured sieve-element. Singh and Srivastava (1972) observed in corn phloem that dictysomes are numerous and produce abundant vesicles of the smooth-walled type which probably fuse with the plasmalemma. Coated vesicles were also reported by these authors in the differentiating sieve-elements.

Changes of the dictyosomes are supposed to be related to the structural changes of endoplasmic reticulum in some way or the other.

The ribosomes can no longer be found in the cytoplasm of the sieve-elements at early stage of their differentiation (Buvat, 1960; Duloy et al., 1961).

Endoplasmic reticulum was observed by a number of workers both in the developing and developed sieve-elements (Buvat,

1960; Duloy et al.; 1961; Esau and Cheadle, 1962a, b; Esau, Cheadle and Risley, 1962; Falk, 1962; Esau, 1963).

According to Esau et al. (1962) these endoplasmic reticulum are supposed to be concerned with the callose formation and the differentiation of the sieve-plates. During further development of sieve-element, these (ER) tubules appear to disintegrate and only isolated vesicles are seen in matured sieve-tube members (Buvat, 1960; Kollmann, 1960; Esau and Cheadle, 1962a, b; Esau, 1963).

Mehta and Spanner (1962), and Falk (1964) have, however, proved the existence of highly differentiated lamellar system in parietal cytoplasm of fully developed sieve-tube members but these are rare.

Singh and Srivastava (1972) noticed that the ER occurs in greater amount in a differentiating sieve-element alongwith the ribosomes and dictyosomes than in the matured one. They further showed that a matured sieve-element shows a loss of tonoplast, degeneration of the nucleus, loss of ribosomes, dictyosomes and microtubules.

Mitochondria :

Relatively little is known about the granular bodies found in sieve-element cytoplasm.

There has been little investigation regarding the presence of mitochondria in sieve-elements. Salmon (1946) for the first

time described some bodies in the cytoplasm of sieve-elements, which she identified as mitochondria. These granular bodies, according to her, vary in size and number with the species. McGivern (1957) identified mitochondria in the sieve-element cytoplasm of Helianthus, Nicotiana, Cucurbita, Gossypium and Beta vulgaris under the light microscopy. She also observed some changes in the state of mitochondria in relation to the age. She found most of the bodies to be spherical but some rod-shaped mitochondria also were observed in the early developmental stages of Cucurbita. She observed them in all the above five species in all the stages of development of the sieve-elements.

Mitochondria are found only in the cytoplasm (McGivern, 1957). In the young sieve-element they are dispersed throughout the cell whereas at later stages they are observed only in the parietal cytoplasm.

The size of the mitochondria in the sieve-element is fairly constant for each species (McGivern, 1957).

The reports regarding the occurrence of mitochondria in matured sieve-elements are conflicting. Buvat (1960) described them to be devoid of contents. Ziegler (1960) on the contrary recognized no intact mitochondria in Heracleum sieve-elements. Hohl (1960) who also could not recognize them in Datura sieve-elements, made an erroneous assumption that during the division of sieve-element mother cell most of the mitochondria migrate into the companion cell.

However Kollmann (1960) found mitochondria with double outer membranes, tubular inner membranes having a rather loose inner structure in the matured sieve-element of Passiflora, under the electron microscopic studies. Duloy et al. (1961) also observed them in matured sieve-elements of Cucurbita pepo although rarely. Kollmann and Schumacher (1961) reported their occurrence in dormant but still nucleate sieve-element of Metasequoia and also in the fully differentiated cells. Esau and Cheadle (1962) concluded during their studies on Cucurbita, that although they are present in matured sieve-elements, they are modified during maturation of the element. Previously Buvat (1960) in Cucurbita and Hohl (1960) in Datura also reported such modification. However the scarcity of mitochondria as reported by Duloy et al. (1961) and Hohl (1960) is only apparant.

The main degenerative changes described by Oberling (1959), DeRobertis, Nowinski and Saez (1960), and Rouiller (1960) are the fragmentation, swelling, accumulation of materials (except water) and fusion.

According to Singh and Srivastava (1972) the mitochondria show least changes, in matured sieve-elements (in fully differentiated sieve-element they still remain intact but have somewhat swollen cristae).

The presence of mitochondria in sieve-elements plays an important role in translocation in phloem (Ullrich, 1961). Since it is a well-known fact that energy requiring processes in the

cell depend directly or indirectly on mitochondria (Hackett, 1955; DeRobertis et al., 1960), it is understood that mitochondria must be present in sieve-elements and they are functioning also. Rouiller (1960) had shown a close relation between the cytochrome quantity and abundance of cristae in mitochondria. Thus the evidence that the abundance of internal membranes in mitochondria is associated with a high rate of enzymatic activity is strongly supported (Key, Hanson & Bills, 1960). At the same time the richness of mitochondria in companion cells and on the other hand the close association of the companion cell with that of the sieve-element also supports the concept that nucleate cells and the sieve-elements are combined into a functional unit (Weatherley, Peel & Hill, 1959).

Plastid :

Briosi (1873) during his studies on a number of dicotyledons found starch grains in the sieve-elements. A little later Russow (1882) and Fischer (1886) also observed the presence of starch in the sieve-elements of many dicotyledons and in a few monocotyledons. This is an indirect indication of the occurrence of plastids in sieve-elements. However, McGivern (1957) and Kollmann (1960) gave the assumption that the starch grains observed by Briosi and ohters in sieve-elements have come from the plastids as a result of the unfavourable influences during preparation.

It was Strasburger (1891) who gave the direct evidence for the occurrence of plastids in sieve-tubes. He observed them in the parietal cytoplasm of sieve-tubes in a number of plants including *Vitis*, *Bambusa* and members of Magnoliaceae, Ranunculaceae, Nymphaeaceae and Tiliaceae and named them as "Starkebinder". Esau (1934, 1941) and McGivern (1957) also reported plastids in sieve-tubes of *Beta*. Recently Ervin and Evert (1970) confirmed the presence of plastids in sieve-tubes of *Polygonatum canaliculatum* and *Typha latifolia*.

Regarding the nature of the sieve-tube plastids Russow (1882) found them to be proteinaceous. McGivern (1957) confirmed Russow's report.

Under the electron microscopic studies the structure and distribution of plastids in the sieve-tubes have been observed more carefully. Several workers (Hohl, 1960; Kollmann, 1960; Mehta and Spanner, 1962; Esau, 1963) observed the presence of plastids with poorly organized internal matrix almost with starch grains of different shape and size, in well-developed sieve-tubes of different angiosperms. There are a number of reports based on electron microscopic observations regarding the presence of plastids in the sieve-elements of different plants viz., Behnke (1967) in *Dioscorea*; Cronshaw and Esau, (1968) in *Cucurbita*; Zee (1968) in *Pisum*; Behnke (1969) in *Arum*; *Luzula scirpus* and *Tacca*; Evert and Deshpande (1969) in *Ulmus*; Palevitz and Newcomb (1970) in *Phaseolus*.

Regarding the structural variation of the sieve-tube plastids, the electron microscopic studies have given the confirmatory evidences as it had been suggested previously by light microscopic studies. According to Behnke (1971), all the sieve-tube plastids could be classified into two main types, 'S' type and 'P' type. 'S' type plastids contain only starch and have been found in a great number of dicotyledons whereas 'P' type contains elaborate protein inclusions and often also starch.

Nucleus :

The well known property of the sieve-element protoplast is its lack of a nucleus at functional maturity. The sieve-element, when young, resembles other meristematic cells in having a more or less vacuolated protoplast with a conspicuous nucleus. The loss of the nucleus occurs during the differentiation of the cells. This characteristic feature of the nucleus was first reported by Wilhelm (1880) and confirmed latter by the reports of a number of workers (Janczewski, 1881; Russow, 1882a; DeBary, 1884; Strasburger, 1882, 1887, 1891). This report of the absence of nucleus was not only confined to the higher vascular plants (Strasburger, 1891; Poirault, 1893; Artschwager, 1924; Crafts, 1932, 1933, 1934; Esau, 1934, 1935, 1938; Abbe & Crafts, 1939; Salmon, 1946, 1947) but also extended upto the vascular cryptogams like the members of Laminariaceae (Will, 1884; Parrot, 1889).

The process of nuclear-disintegration as described by Crafts (1934), Esau (1938), Abbe & Crafts (1939), occurs in two stages involving the enlargement of the nucleus followed by the loss of of its chromaticity. Further Esau & Cheadle (1965) studied the process in detail and observed that first there is a loss in chromaticity of the nucleus, followed by the disappearance of the nuclear envelope. In contrary Shah and James (1968) reported in Nemtunia oleracea that the nuclear-membrane disappears prior to the disintegration of the nuclear material. In their recent communication however, Behnke (1969a, b,), Shah and Jacob (1969), Ervin & Evert (1970) noted the similer process of nuclear-disintegration as has been reported earlier by Esau and Cheadle (1965).

In certain monocots like Arum maculatum, Convallaria majalis (Salmon, 1946, 1947) and in Vanilla planifolia (Segonzac, 1958), the nuclear-disintegration is slower than in dicotyledons.

Recent investigations of Shah & Daniel (1971) had shown three types of nuclear-disintegration in Pennisetum typhoides.

The early disintegration of the nucleus had also been confirmed by the electron microscopic studies of Buvat (1960), Kollmann & Schumacher (1961), Esau & Cheadle (1962a), Esau, Cheadle & Risley (1962) and Mehta and Spanner (1962).

The exact time of nuclear-disintegration varies in different plants (Esau, Cheadle & Risely, 1962). In Cucurbita maxima as

reported by the above workers, the nuclear disintegration takes place before the development of the perforation in the sieve-elements. However, others noted that the nuclear disintegration occurs after the development of the sieve pores (Kollmann & Schumacher, 1969; Wark and Chambers, 1965; Evert et al., 1966).

In contrary to the above findings, reports are there regarding the presence of nuclei in mature sieve-elements. The first report in this respect is that of Fischer (1886) in Urtica. Other workers also reported them in matured sieve-elements, viz., Lecomte (1889) in Cucurbita, Impatiens, Vitis and Macropiper and Scott & Brebner (1889) in Strychnos.

There are several recent reports of the occurrence of nuclei in matured sieve-elements.(Table 1).

T A B L E -I

OCCURRENCE AND MORPHOLOGY OF NUCLEI IN MATURE SIEVE - ELEMENTS

S.NO.	SPECIES	MORPHOLOGY	REFERENCE
1.	*Isoetes*	Necrotic	Paolillo (1963)
2.(a)	*Juniperus virginiana*	Necrotic	Evert and Alfieri (1965)
(b)	*Larix decidua*	"	"
(c)	*Picea mariana*	"	"
(d)	*Pinus banksiana*	"	"
(e)	*Pinus resinosa*	"	"
(f)	*Pinus strobus*	"	"
3.	*Pinus strobus*	"	Murmanis & Evert (1966)
4.	*Pinus strobus*	"	Srivastava & O'Brien (1966)
5.	*Pinus pinea*	"	Wooding (1966)
6.	*Secale*	"	O'Brien and Thimann (1967)
7.	*Neptunia oleracea*	"	Shah and James (1968)
8.	*Ulmus americana*	Normal	Evert et al. (1969)
9.	*Austrobaileya scandens*	Necrotic	Srivastava (1970)

10.		*Smilax hispida*	Normal	Ervin and Evert (1970)
11.		Some of the palms	Necrotic	Parthasarthy (1966) (Quoted by Evert et al., 1970)
12.	(a)	*Metasequoia glyptostroboides*	Normal	Evert, Davis, Tucker and Alfieri (1970)
	(b)	*Sequoia sempervirens*	Normal	"
	(c)	*Taxodium distichum*	Normal	Evert et al. (1970)
	(d)	*Acer negundo*	Clear to dense spherical body	"
	(e)	*Acer saccharinum*	Clear spherical body	"
	(f)	*Cornus racemosa*	Clear spherical body	"
	(g)	*Cornus stolonifera*	Dense spherical to elongate body	"
	(h)	*Juglans nigra*	Relatively dense spherical body	" "
	(i)	*Populus tremuloides*	Relatively clear spherical body some-times swollen	" "
	(j)	*Quercus alba*	Clear spherical body	"
	(k)	*Rhus glabra*	Clear to dense spherical body	"
	(l)	*Robinia pseudoacacia*	Normal to dense spherical body	"
	(m)	*Tilia americana*	Clear crumpled body	"

(n)	Ulmus americana	Normal to clear spherical body, sometimes swollen	Evert et al. (1970)
(o)	Vitis riparia	Normal to clear spherical body	
13.	Isoetes	Amorphous, granular, chromatin with nuclear envelope.	Kroutrachve and Evert (1974)

In some plants the nucleolus was reported to persist even after the disappearance of the nucleus in the sieve-elements. Engard (1944) for the first time reported the extrusion of the nucleoli in Rubus and interpreted these intrusions as slime bodies. Similer inclusions were also reported earlier by Lecomte (1889), who described them as albuminous globules. Crafts (1939) observed these inclusions in some plants like Casuarina, Eucalyptus, Gossypium and described them as sculptured spherical bodies. Esau (1947) confirmed the Engard's report of extrusion of nucleoli in Rubus & also observed the same case in Eucalyptus and Gossypium. She also noted that the extruded nucleoli are distinct from the slime bodies and that both types of inclusions occur in the same element. Zahur (1959) reported extruded nucleoli in 41 species of dicotyledons. Andrews (1967) also had shown them in Populus deltoides, Quercus alba, Salix nigra and Tilia americana in the form of persistent spherical structures and interpreted them as extruded nucleoli. Recently Evert et al. (1970) also had reported the extruded nucleoli consisting of rod like components in quasi-crystelline aggregates. But Singh and Srivastava (1972) could not find extruded nucleoli in corn phloem during their ultrastructural studies.

Ontogeny of sieve-element :

Janczewski (1881) studied the ontogeny of sieve-elements in various groups of vascular plants representing the dicotyledons, gymnosperms, pteridophytes and monocotyleons and concluded that it is almost uniform in all these cases. He

recognised four distinct stages :

(1) Evolutionary stage,

(2) active stage,

(3) transitional stage and

(4) the passive stage.

The first stage starts from the cambial state to the development of perforations in sieve-plate, the second is the period when the connecting strands are clear in the sieve-plate, in the third large masses of callose develope on sieve-plate and in the last there is a complete disappearance of callose and death of the element followed by the removal of cytoplasm.

Esau (1938a) also recognized four distinct stages during ontogeny of sieve-elements in different dicotyledons (Vitis and Cucurbita), viz :

1. Developmental
2. Mature
3. Transitional and
4. Degenerative stage.

Stage 1. (developmental)

In the developmental stage the cells derived from the procambium have many pits traversed by plasmodesmata on the longitudinal walls but prominent on the end walls. The primary pit-fields of these elements are the future sieve-areas. When the cell expands due to turgidity stretching and reduction in

thickness of the pit-closing membrane result leading to the formation of pores (Abbe & Crafts, 1939). But the rest of the wall thickens by cellulose deposition resulting in the nacré thickening of the wall. Soon after this, callose appears around the pores where the plasmodesmata pass through the wall. The protoplast of these elements is just like that of parenchyma having nucleus, vacuole and embryonic plastids. Cytoplasm shows the usual streaming. Appearance of slime bodies clearly marks the beginning of the specialization of the sieve-tube. The nucleus and the slime-bodies disintegrate and the rate of cytoplasmic streaming gets changed. According to Abbe and Crafts (1939) cytoplasmic streaming increases at first, slows down and then stops completely. Now the cytoplasm occupies a parietal position and the elements enter the mature stage. Starch grains if present enter the vacuole and the cytoplasm becomes permeable.

Stage 2. (Mature)

The young sieve-element is now a thick-walled elongated cell with a parietal cytoplasm and a large central vacuole. The connecting strands and amount of callose which are small at the beginning of maturity, continue to develop gradually. Towards the end of maturity the nacré wall starts losing the thickening and the callose amount is very evident on the sieve-plate.

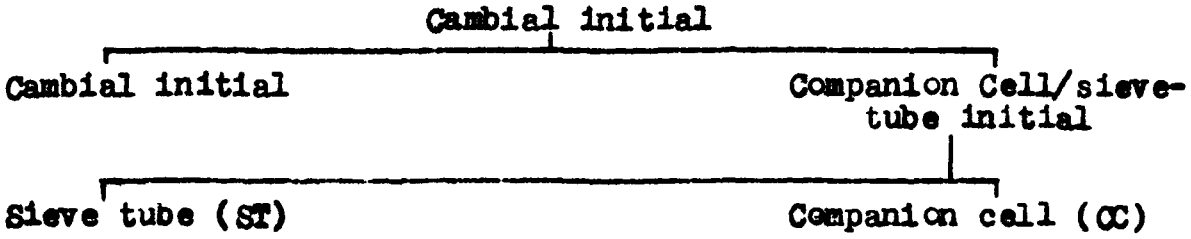
Stage 3. (Transitional)

As the sieve-element ages, the amount of callose increases within the pores, constricting the passing cytoplasmic strands. The callose now forms definitive one.

Stage 4. (Degenerative)

The definitive callose dissolves and cytoplasm dies off at this stage resulting in the opening of the pores in the sieve-plate. As a dead cell with unlignified walls, the sieve-element is unable to resist the pressure of the adjoining living cells and soon gets obliterated or becomes crushed.

Northcote and Wooding (1969) described the ontogeny of sieve-elements during their electron microscopic studies on sycamore. They distinguished 4 distinct stages as follows :



1. a. Walls become secondarily thickened.
   b. Golgi bodies present producing vesicles.
   c. Endoplasmic reticulum sheathing plastids.
   d. Simple plasmodesmata with CC.
   e. Callose formed around plasmodesmata with CC on ST side.
   f. Nucleus large, normal structure.
   g. Loose bundles of fibrils (80-100 A° diameter) found in Cytoplasm.
   h. Slime body appears with fibres (190-210 A° diameter).
   i. Endoplasmic reticulum closely applied in limited profiles on both sides of future sieve-plates.
   j. Endoplasmic reticulum profiles found at plasmodesmata between ST and CC.
   k. Smooth endoplasmic reticulum formed and it associates in aggregates.

II. a. Callose formed underneath endoplasmic reticulum at sieve-plate.

b. Golgi bodies decrease in number.

c. Tonoplast comes away from the peripheral cytoplasm and profiles of this membrane can be seen in the vacuolar sapce.

d. Nucleus takes up less of the electron stains (Uranium and Lead)

e. Less ribosomes free in Cytoplasm and on endoplasmic reticulum.

f. Aggregates of smooth membranes in the form of hexagonally packed vesicles found at the CC/ST pores.

III. a. Plasmodesmata in the sieve-plate widen within the wall at the level of the middle lamella.

b. The bone-shaped mass of callose situated around both ends of the sieve-plate pore are reduced in amount and callose comes to line the pore.

c. Tonoplast breaks up and vacuolar space mixes with degenerating cytoplasm.

d. Golgi bodies reduced in number.

e. Ribosomes reduced in number.

f. Remaining endoplasmic reticulum collected into the organized

aggregates.

g. Plastids lose sheath of endoplasmic reticulum and all material except starch grains.

h. Additional pairs of membranes form at the nuclear surface and a general dispersion of the nuclear contents occurs and the nucleoli disappear.

i. The slime body disperses into the cell space to give fibrils (80-100 A^{o} diameter) with an axial repeating pattern.

IV. a. Fully pierced pores lined with callose at sieve-plate. Cell lumen filled with diffuse network of fibrils. At the wall lamellar stacks of membranes associated with the hexagonally packed vesicles occur.

b. The organized aggregates of endoplasmic reticulum disintegrate.

c. Plastids with starch grains only.

d. No golgi bodies, no riboromes.

e. Occasional small mitochondria found at the cell membrane.

f. Plasmalemma persists.

Structure and development of companion cell :

The sieve-tube members are associated with a specialized parenchyma cell, the companion cell. After the discovery of companion cell by Wilhelm (1880) it was investigated by many workers (Janczewski, 1881; Russow, 1881, 1882a; Lecomte, 1889; Strasburger, 1891) that they are the characteristics of dicotyledonae and monocotyledonae. They were regarded as a general feature of metaphloem and secondary phloem of angiosperms (Esau, 1939). They were found to be absent in the protophloem of angiosperms specially in roots (Léger, 1897b; Chauveadd, 1895, 1900; Chang, 1935; Esau, 1935).

The companion cells may occur on different sides of their sieve-tube element or they may form continuous longitudinal series on one side of it as was reported by Strasburger (1891) in some herbaceous dicotyledons and in many monocotyledons. But according to him in other plants, the companion cells of different sieve-tube elements were not in contact with each other.

The walls between the companion cells and sieve-elements are either uniformly thin or having primary pit-fields (Esau, 1965). These walls are traversed by plasmodesmata which are often branched at the companion cell side as was revealed by Esau & Cheadle (1962b) under electron microscope.

The protoplast of matured companion cells stains deeply than that of ordinary parenchyma cells. It had been thought that this staining property was due to the presence of a subs-tance similar to the slime of the sieve-tube elements. Esau (1950, 1948) found slime-bodies in the companion cells of Vitis and observed that with the dispersal of this slime, the protoplast stained more intensely. They also had small vacuole. Early workers (Wilhelm, 1880; Russow, 1882a; Fischer, 1885b; Lecomte, 1889; Strasburger, 1891) observed that they do not contain starch but have granular cytoplasm with well differentiated nuclei. Later on Esau (1965) also recognized that they do not form starch but may contain leucoplasts or chloroplasts. They retain their mitochondria, dictyosomes and endoplasmic reticulum at maturity (Esau & Cheadle, 1961, 1962b).

Ultrastructural study of the matured secondary phloem of Tilia americana (Evert and Murmanis, 1965) had revealed that plastids are absent from the companion cells but the recent report of Evert & Deshpande (1971) had shown that they do occur in companion cells.

Although the companion cells form the chracteristic feature of the angiosperm phloem, there is no comprehensive data regarding their distribution in this group. According to Esau (1939) they are not present in the protophloem of angiosperms. Bailey and Swamy (1949) had, however, reported that they are absent in the primitive woody dicotyledons.

Detailed studies on the secondary phloem of different plants had revealed that a few sieve-tube elements had no companion cells (Cheadle & Esau, 1958; Evert, 1960, 1963a).

The gymnospermous sieve-cells had no companion cells in the sense of Wilhelm (1880), but Strasburger (1891) found some cells in this group of plants associated with the sieve-tube elements by sieve-pit and they obliterated simultaneously with their sieve-tubes. He held these cells to be analogous to the companion cells of angiosperms and named them albuminous cells. Recently Srivastava (1963) had reported starch to be present in these cells. No report of presence of slime in albuminous cells had come during light microscopic studies. However, Murmanis & Evert (1966) had recognized slime in albuminous cells of Pinus strobus.

In conifers and Ginkgo certain ray and phloem parenchyma cells are closely associated with sieve-cells morphologically and physiologically (Esau, et al., 1953; Grillos and Smith, 1959; Srivastava, 1963a, b). These parenchyma cells stained deeply with cytoplasmic stains, albuminous cells (Strasburger, 1891). Like those of companion cells, the albuminous cells lack starch and die with the disorganization of the sieve-cell. Thus the relation between albuminous cells and the sieve-cells resembles in angiosperms except that these are different ontogenetically having different mother cells (Srivastava, 1963b). Sclerification of the companion cells in old phloem

of Tilia has also been reported by Evert (1963c).

Companion cells are reported to be lacking in vascular cryptogams (Strasburger, 1891; Poirault, 1893; Palm, 1936; Esau, 1965).

Ontogeny :

The close association between the companion cell and sieve-tube is not only evidenced in their common origin but also in their simultaneous death and obliteration (Bliesenick, 1891; Strasburger, 1891; Léger, 1897b; Esau, 1936, 1938a). In the formation of companion cell the precursor meristematic cell divides longitudinally to form one larger and the other shorter cell, the former differentiates into sieve-tube member while the latter one, with or without further divisions differentiates into companion cell. They are sometimes as long as the sieve-tube members but often they are shorter than the former. The number of companion cells associated with a given sieve-tube member varies from one to many in different species and also within the same plant (Cheadle & Esau, 1958; Zahur, 1959).

Structure and development of phloem parenchyma :

Phloem parenchyma and companion cells are the two constant components of dicotyledonous phloem. These two were named by separate terms namely bast parenchyma and cambriform cells by

the early workers (Nägeli, 1858; Russow, 1872). Later Wilhelm (1880) called the cambriform cells as companion cells. Since then the term cambriform was dropped.

Phloem parenchyma forms the characteristic feature of the primary and secondary phloem of all the vascular plants (Esau, 1965).

Parenchyma cells present in the primary phloem are elongated and are oriented in the same direction as the sieve-element, while in the secondary phloem it occurs in two systems, the axial and ray system.

The walls of phloem and ray parenchyma cells have primary pit-fields which interconnect axial and ray parenchyma cells with one another and within themselves (Esau, 1965).

In the active phloem, the phloem parenchyma cells have primary unlignified walls but after the cessation of activity they may become sclerified or may not (Esau, 1965).

The phloem parenchyma cells can easily be differentiated from the companion cells by their small amount of cytoplasm with large vacuoles (Esau, 1939). The phloem parenchyma are concerned with many activities such as the storage of organic food materials and accumulations of tannins and resins (Esau, 1939). Certain phloem parenchyma cells in secondary phloem accumulate crystals or resins and then die (Strasburger, 1891; Abbe and Crafts, 1939).

Esau (1947) reported that structures similer to slime bodies occur in some phloem parenchyma cells of Eucalyptus. Recently P-protein has been reported to occur in the secondary phloem parenchyma cells of Parthenocissus inserta and Vitis riparia by Davis and Evert (1970).

Structure and development of phloem fibres :

Fibres form a frequent component of phloem. Many dicotyledons have a group of fibres, pericyclic (Morot, 1885; Eames and MacDaniels, 1947) or primary phloic (Esau, 1938a, 1943, 1948) on the periphery of the vascular system. According to Esau the fibres occurring at the periphery of the vascular system in many dicotyledonous stems are in fact, primary phloem fibres and not pericyclic in origin.

The secondary phloem fibres arise from the fusiform cambial cells as a component of the axial system, they may elongate by apical intrusive growth but still they are shorter than the primary phloem fibres of the same plant (Esau, 1965; Kundu, 1942).

According to Lecomte (1889) and Strasburger (1891) the fibre wall in primary phloem is thicker than those in the fibres of secondary phloem. Matured fibres, may be primary or secondary, are dead cells. They have got simple pits, sometimes slightly bordered. In some plants they are lignified, in others this is not the case (Esau, 1965). Septate secondary phloem fibres have been reported in Vitis (Strasburger, 1891; Esau, 1948) and in

Dalbergia (Ghouse & Yunus, in press). Fibres occur in the protophloem and not in the metaphloem (Esau, 1950).

In some plants the secondary phloem fibres mature in the conducting phloem and specialized as mechanical elements (e.g. Tilia) while in others (Prunus, Schneider, 1945) they remain alive in conducting phloem and mature into fibres only after the cessation of function of sieve-elements.

There are reports of the occurrence of living fibres which store starch and remain alive even after maturity e.g. septate fibres of Vitis (Esau, 1948). Their continued growth in the nonconducting region by apical intrusive growth had also been observed in certain plants (Kundu, 1942; Liese and Parameswaran, 1972; Ghouse and Yunus, in press).

Ontogeny :

The primary phloem fibres are ontogenetically related with the sieve-elements, i.e. they arise from the same part of the procambium as the sieve-element (Esau, 1943, 1948).

Fibres originating during primary growth have a different kind of development than those formed in the secondary tissues. Primary fibres elongate before the organ has elongated while the associated cells are still dividing. This is followed by the apical intrusive growth. In contrast, secondary fibres originate in the part of the organ that has ceased to elongate and they can

increase in length only by intrusive growth (Esau, 1965). Sometimes, due to different type of growth in primary and secondary fibres, the primary may attain greater lengths than the secondary as in Cannabis where the primary phloem fibres attain an average of 13 mm, whereas the secondary about 2 mm (Kundu, 1942).

The apices of the fibres remain thin walled and rich in cytoplasm for a long time. They may become serrated and forked during their adjustment among the adjecent cells. In flax the phloem fibres have been found growing at both apices, upward and downward (Schoch-Bodmer and Huber, 1945, 1951).

The manner of secondary wall development is quite complicated in case of fibres. The deposition of secondary walls begins after the primary wall completes its increase in surface (Esau, 1965).

When the primary fibres elongate by symplastic growth, they remain thin-walled. During this, the entire fibre wall increases. After this, during the apical growth stage, the apices of the cells remain thin, while the median portions of the cells, which have completed their elongation, begin to form secondary walls. In Linum and Boehmeria (Aldaba, 1927; Anderson, 1927), the secondary wall of the fibres develops in the form of distinct lamellae. Similar type of development had been observed by a number of workers in other plants too (Ghosh, 1943; Kundu, 1942; Esau, 1943, 1948; Artschwager, 1943; Schneider, 1945).

Vascular cambium and its behaviour :

Growth in thickness due to the lateral meristem, the vascular cambium, is called secondary growth and the tissues thus produced are termed as secondary tissues. These tissues constitute the secondary body of the plant, in the stems and roots, and sometimes even in the petiole and main veins of the leaves.

They develop from the secondary meristems i.e. from the vascular cambium and the phellogen or cork cambium.

In monocotyledons and some of the lower vascular plants all the cells of the procambium are consumed in the differentiation of xylem and phloem. But in almost all the dicotyledons and gymnosperms, a portion of the procambium remains meristematic even after the completion of primary growth and differentiation into the cambium of secondary body. It is lateral meristem. The cambium that arises within the primary vascular tissue is called as fascicular cambium and the cambium that develops from the inter fascicular parenchyma is termed as interfascicular cambium (Esau, 1965).

In most dicotyledons and gymnosperms, the cambium develops between the primary xylem and phloem and produces secondary

xylem centripetally and secondary phloem centrifugally (Esau, 1965). However, this is not the case in some plants e.g. in Chenopodiaceae, where the secondary growth is anomalous (Fahn, 1967). Important works regarding the structure and manner of cell division in cambium had been done by Bailey (1920a, b, 1923, 1930), Bannan (1950, 1951a, b)Bannan and Whalley (1950), Newman (1956) and Wilson (1964).

The vascular cambium is usually composed of two types of elements viz., elongated elements with tapering end walls -- the fusiform initials, and the nearly isodiametric relatively small ray initials. The fusiform initials give rise to the longitudinally oriented elements in an organ, such as the tracheary elements, fibres, xylem and phloem parenchyma and the sieve-elements while the ray initials give rise to the cells of horizontally oriented vascular rays.

On the basis of the cellular agrangement generally two types of cambia are recognized (Bailey, 1930; Eames & MacDaniels, 1947; Esau, 1965; Fahn, 1967; Ghouse & Yunus 1972; Ghouse et al., 1973) viz., non-stratified and stratified.

During active growth in the cambium the initials and their immediate derivatives form a zone of similar unexpanded meristematic cells, the cambial zone. The cells in the cambial zone are arranged in radial series.

There are different opinions of authors in referring the term cambium to the single layer of initials or to the whole

cambial zone consisting of the neighbouring cells derived from them. The authors who consider the whole population of undifferentiated cells, situated between xylem and phloem as the cambial cells are Bannan (1955) Newman (1956), Wilson (1964), Catesson (1964) and Ghouse et al. (1973). While others have conceived in the occurrence of the single layer of cambial initials (Bailey, 1943; Eames & Mac Daniels, 1947; Foster, 1949; Esau, 1965).

The cambial initials divide periclinally and anticlinally in a longitudinal plane. As a result of the periclinal (tangential) divisions, which are more numerous, new cells are added to the secondary xylem and phloem, the former towards the interior of the axis and the latter towards its periphery. The derivatives of each initial therefore arrange in radial rows but this radial seriation may be lost during the growth readjustments of the cells.

New ray initials arise from the fusiform initials or from their segments. A ray may be one cell wide and one cell high in the beginning (uniseriate ray), later the initial divides. The ray thus increases in height and may increase in width also if the plant has got the characteristic multiseriate rays. Some workers (Brown, 1915; Evert 1961) reported that new ray initials may be cut of the apices or from the side of the fusiform initials. Cumbie (1963) reported in a herbaceous species of Hibiscus that the rays are derived by transverse divisions, from the fusiform cells. In certain conifers (Bannan, 1951,

1953, 1956) and dicotyledons (Cheadle & Esau, 1964) there occurs a complicated process involving sub-division of fusiform initials, elimination of some products of these division and transformation of others into ray initials. Rays may increase in width and height by the fusion of two or more groups of ray initials (Braun, 1955). However, the splitting of rays also occurs in some plants but is less common. The phenomenon of loss of initials has been studied in detail in conifers (Bannan, 1951, 1962; Hejnowicz, 1961; Forward & Nolan, 1962) and in dicotyledons (Evert, 1961; Cheadle & Esau, 1964). The loss of fusiform initials is gradual.

Seasonal activity of cambium :

There are plants whose cambium is active for a major part of the year and undergo dormancy only for a few months. This type of activity is the characteristics of tropical plants (Chowdhury, 1940, 1941). On the other hand in temperate plants, the cambium ceases its activity with the onset of unfavourable conditions i.e. in the autumn, and remains dormant till the following spring. In spring, the winter rest period is succeeded by a reactivation of cambium. From the anatomical point of view the commencement of the cambial activity consists of 2 stages 1. the cambial cells become wider radially and 2. the initiation of cell division. With the enlargement of the cambial cells their radial walls become weakened so that a slight force may cause

the peeling off the bark of stems and roots. The separation of the bark from the wood resulting from such a break is called slipping of the bark. The slippage may also occur in later stages due to the increase in number of cells in the cambial zone due to cell division. At this time the separation occurs in the region of young xylem cells which have attained their maximum diameter but which still have thin primary walls (Bailey, 1943; Evert, 1960, 1961).

The important contribution regarding the seasonal activity of cambium in tropical trees is those of Fahn and his coworkers in the Mediterranean and desert regions of Israel, and of Chowdhury on some Indian trees. As has already been mentioned there are plants in which the cambium is active throughout the year and those in which there is a break in duration of the cambial activity.

Fahn (1967) had recognized five categories of plants in Israel, the cambial activity in each commencing at a different period i.e. the categories are based on the time of initiation of cambial activity.

1. In the first category of plants which included woody species namely Retama raetam Webb., Artemisia monosperma Del., Zygophyllum dumosum Boiss. and Reaumuria palaestina Boiss, the cambium is dormant for a long period with the radial growth beginning in early months of winter i.e. between November and January.

2. In the second category which included some trees and shrubs such as Quercus spp., 3 Pistacia spp., Ceratonia siliqua L., Tamarix jordanis Boiss. var. negevensis Zoh., T. gallica L. var. maris-mortui Zoh., and Colligonum comosum L' Her., the cambial activity commences in the spring i.e. March to May. Some of these plants have a marked dormant period while in others as in Ceratonia and the two Tamarix spp., the cambium is inactive for a very short period only and may even remain active throughout the year.

3. The third category of plants which includes only shrubs such as Anabasis articulata Maq. and Salsola rosmarinus Solmlaub., in which the commencement of growth ring production is in February.

4. The fourth category which includes trees of Eucalyptus camaldulensis Dehn. and Tamarix aphylla Karst., in which the formation of the early wood starts either in August (T. aphylla) or in September (E. camaldulensis) i.e. towards the end of the dry summer season.

5. The fifth category includes some trees and shrubs (Acacia tortilis Hayne, A. raddiana Savi, A. cyanophylla Lindl. and Thymelaea hirsuta Endls) in which the wood production is carried out throughout the year.

In this connection the work of Chowdhury on some Indian trees is worth mentioning. He studied the following eleven trees.

of North India viz., Pinus roxburghii, Tectona grandis, Cedrela toona (Chowdhury, 1939 , 1940); Terminalia tomentosa, Michelia champaca (Chowdhury, 1934 , 1936 , 1947, 1953); Acacia catechu, Bombax malabaricum, Albizzia lebbek, Dalbergia sisoo, Shorea robusta and Eugenia jambolana (Chowdhury, 1939 , 1940). In all the spp. the time of the formation of growth rings differs.

In Pinus the commencement of the radial growth occurs in February, i.e. earlier than all the other spp. studied mentioned above. Cedrela toona is the second in this refer-ence whose cambial activity starts in March. Rest of the spp., no matter they are ringporous or diffuse-porous group of trees, begin their radial growth from early June to early July. In all of them the dormancy of the cambium starts from the middle of October to the middle of November.

Factors influencing cambial activity :

The relationship between cambial activity and the activity of the vegetative buds was suggested by many workers (Fraser, 1962; Ladefoged, 1952). The activity of the cambium in many dicotyledons usually begins below the sprouting buds from where it spreads downwards (basipetally). The velocity of this spread varies. In Acer it was found to be rather slow (Cockerham, 1930) while in conifers, in some ring-porous dicotyledons (Priestley, 1930; Wareing 1951) and in some evergreens (Fahn, 1953) the rate is very rapid.

The stimulus for the reactivation of cambium is apparantly, a certain level or combination of growth regulating substances (Gouwentak, 1941). However the initial stimulation of cambial activity is related to the transport of growth substances from the growing buds in basipetal direction (Samish, 1954). The maintenance of cambial activity is independent.

Seasonal development of secondary phloem :

Despite the presence of voluminous literature on cambial activity and xylem production (Grossen Bacher, 1915; Priestley, 1930; Bannan, 1955; Wareing, 1958) comparatively less is known about the seasonal development of the phloem. According to Esau (1965) this might be due to the lack of exacting techniques required for its investigation and to the concept that xylem and phloem production occurs simultaneously or that xylem production preceedes that of the phloem. Further the information on the seasonal development of secondary phloem in tropical plants is much rare as compared to the temperate trees.

Some important early works on the phloem development included those of Janczewski (1881); Russow (1882a) on pine, Strasburger (1891), Knudson (1913), Schneider (1917), Swarbick (1927), on apple, Gill (1932) on Fraxinus, Priestley (1935), Elliott (1935) on Acer psudoplatanus, Esau (1939) on grapevine, Huber (1939) on Abietineae, Schneider (1945, 1952) on peach, cherry and Citrus sinensis, Esau (1948) on Vitis, Holdheide (1951) on Tilia,

Smith (1958) on Douglas-fir.

The important recent work on this regard is given in the Table 2.

T A B L E -2

NAME OF SPECIES	DURATION OF CAMBIAL ACTIVITY INITIATION	CESSATION	DURATION OF PHLOEM PRODUCTION INITIATION	CESSATION	PERIOD OF PHLOEM ACTIVITY	REFERENCE
Acer pseudo-platanus	Late March-early August	Late July-early August	Late March-early April	Sept. - end of Dec.	5 months	Cockerham (1930)
Acer platanoides	-do-	-do-	-do-	-do-	-do-	Huber (1939)
Acacia catechu	Early June-early July	Middle of Oct.-middle of Nov.	-	-	-	Chowdhury (1939, 1940)
Albizzia lebbek	-do-	-do-	-	-	-	-do-
Acer negundo	Late March or early April	Late July-Aug.	Late March or early April	Sept.-end of Dec.	5 months	Tucker and Evert (1969)
Albizia adianthifolia	Cambium is active throughout the year		April - September at maximum		Active Phloem present throughout August	Lawton (1972)
Antiaris africana	-do-		April and Dec. at maximum			-do-
Bombax malabaricum	Early June-early July	Middle of Oct.-middle of Nov.	-	-	-	Chowdhury (1939, 1940)

Bombax buonopozense	Cambium is active throughout the year		April - September at maximum		Active Phloem present throughout August	Lawton (1972)
Cedrela toona	March	Middle of Oct.- middle of Nov.	-	-	-	Chowdhury (1939, 1940)
Carya illinoensis	Late March		Early May			Artschwager (1950)
Celastrus scandens	Early May	Mid August	Early May	Mid Oct.- late Dec.	5½ months	Davis and Evert (1970)
Dalbergia sisoo	Early June- early July	Middle of Oct.- middle of Nov.	-	-	-	Chowdhury (1939, 1940)
Eugenia jambolana	Early June- early July	Middle of Oct.- middle of Nov.	-	-	-	-do-
Holarrhena floribunda	Cambium is active throughout the year		April - September at maximum		Active phloem present throughout August	Lawton (1972)

Michelia champaca	Early June-early July	Middle of Oct.-middle of Nov.	-	-	-	Chowdhury (1934, 1936, 1947, 1953)
Pinus roxburghii	February	Middle of Oct.-middle of Nov.	-	-	-	Chowdhury (1939, 1940)
Pyrus communis	Late March-early April	Late July-early Aug.	Early April	Late Sept. or early Oct.-late Nov. or December.	5½ months	Evert (1960)
Pyrus malus	-do-	-do-	-do-	Late Sept. or early Oct.-late Nov.	5 months	Evert (1963)
Pinus strobus	End of March or early April	Early Aug.	End of March or early April	Late June-end of Oct.	3 months	Alfieri and Evert (1968)
P. resinosa	-do-	-do-	-do-	Mid July-late Oct.	3½ months	-do-
P. banksiana	-do-	-do-	-do-	Mid Aug.-Late Oct.	4½ months	-do-
Populus termuloides	Late March or early April	July or August	Late March or early April	Late Sept. or early Oct.-December	5½ months	Davis and Evert (1968)

Parthenocissus inserta	Early April – Early August	Early April	Early Oct.- mid. Dec.	6 months	Davis and Evert (1970)
Robinia pseudoacacia	-do- – Early Sept.	-do-	Late Sept.- late Nov.	5½ months	Derr & Evert (1967)
Ricinodendron heudelotii	Cessation of cambial activity in dry period i.e. August	Lowest amount in Aug. (dry period)		-	Lawton (1972)
Shorea robusta	Early June- early July – Middle of Oct.- middle of Nov.	-	-	-	Chowdhury 1939, 1940)
Sterculia tragantha	Cambium is active throughout the year	April - September at maximum		Active phloem present throughout August	Lawton (1972)
Tectona grandis	Early June- early July – Middle of Oct.- middle of Nov.	-	-	-	Chowdhury (1939, 1940)
Terminalia tomentosa	-do- – -do-	-	-	-	Chowdhury (1934, 1936, 1947, 1953)
Tilia americana	Early May	Mid May	September	3½ months	Evert (1962)
Tectona grandis	Cessation of cambial activity in dry period i.e. August	Greatest amount of active phloem in April - Oct. and lowest amount in Aug. (dry period)		-	Lawton (1972)

Ulmus americana	Mid April	Late July-August	Mid or late April	Oct.-mid. Dec.	5 months	Tucker (1968)
Vitis labrusca	Mid June	Early August	Mid June-early July			Knudson (1916)
Vitis vinifera	-do-	-do-	-do-			Esau (1948)
Vitis riparia	-do-	-do-	-do-	Previous season's sieve-elements become non-functional in late July-early August. Current season's sieve-elements develop dormancy callose during last ½ of October.		Davis and Evert (1970)

The systematic studies on the seasonal development of secondary phloem in certain tempertate trees carried out by Evert et al. in the last one decade had brought many things to light. In this connection the work on seasonal cycle of phloem development and the relationship between phloem and xylem differentiation on the 4 woody dicotyledonous species (Pyrus communis, Evert, 1960; Pyrus malus, Evert, 1963; Robinia pseudoacacia, Derr and Evert, 1967; Populus termuloides, Davis and Evert, 1968) studied by them produced interesting results, according to which in all the four species (1) all the sieve-elements produced in a given season remained functional only for that season (they did not continue to function in the next).
(2) the first functional sieve-elements differentiated in spring from cells which were produced at the close of the previous season and overwintered in an undifferentiated state (Precursor phloem).
(3) The time of differentiation of the above sieve-elements (Precursor phloem) coincided with the time of reactivation of cambium.
(4) Phloem differentiation preceeded xylem differentiation in spring.

Further, Evert and his associates discovered the occurrence of two patterns of differentiation of xylem and phloem in some temperate trees. In the ring-porous spp. (Celastrus scandens, Davis and Evert, 1966; Robinia pseudoacacia, Derr and Evert, 1967) phloem and xylem differentiation begins almost at the same time,

whereas in case of diffuse-porous spp. (Pyrus communis, Evert, 1960; Pyrus malus, Evert, 1963; Populus tremuloides, Davis and Evert, 1968) phloem and xylem differentiation is separated by 4 to 6 weeks with the exception of Tilia americana (Evert, 1962) in which both xylem and phloem differentiated almost at the same time. Vitis spp. (Knudson, 1916; Esau, 1948; Davis and Evert, 1970) were found to be similar in this respect to Tilia.

Lawton and Lawton (1971) and Lawton (1972) made a detailed study on the seasonal variations in the secondary phloem of some forest trees from Nigeria.

Functioning period of secondary phloem :

In most dicotyledons the functioning part of the phloem is restricted to that secondary phloem that is produced in the last growth season (Fahn, 1967, p-329). However in some, almost all the phloem which was produced in the previous season ceases to function before the cambium begins to produce new phloem in the current season. Tilia and Vitis were found to be two exceptions in which the phloem remains active for a number of years, upto 10 years in T. cordata Mill (Holdheide, 1951) and from 1-5 years in T. americana L. (Evert, 1962). In Vitis (Esau, 1948; Bernstein and Fahn, 1960) it remained active for two seasons.

In plants with included phloem e.g. Bongainvillea and some woody spp. of Chenopodiacea, the phloem remained active for many years (Fahn, 1967). In most conifers the activity of the phloem

elements is restricted to a single growing season (Esau, 1965).

The presence of functional phloem in the whole of the year in case of dicotyledons had been reported in a few species only. In most of the plants studied the sieve-elements become non-functional the same season in which they were derived from the cambium (Esau, 1939, 1950; Huber, 1939; Holdheide, 1951). Many workers had demonstrated that in some woody dicotyledons, occasionally small sieve-elements remain functional until new phloem was produced in spring. In some spp. viz., pecan (Artschwager, 1950), yellow birch (Wilcox et al., 1956) and some conifers especially in Pinus strobus (Strasburger, 1891; Brown, 1915; Abbe and Crafts, 1939; Wilcox et al., 1956; Grillos and Smith, 1959; Srivastava and O'Brien, 1966) the later formed sieve-elements at the close of the season underwent only partial differentiation and they over wintered in the same condition. However, Alfieri & Evert (1968) could not find any evidence for the presence of such partially differentiated overwintering sieve-elements in Pinus at the light microscopic level. Cockerham (1930), Elliott (1935) and Huber (1939) also did not find any differentiating or partly differentiated sieve-elements in winter in case of Acer. In addition they reported the complete absence of the functional sieve-elements (matured out of the precursor phloem) at the time of cambial reactivation in spring.

Contrary to the above in several spp. viz., Pyrus communis P. malus (Evert, 1960, 1963), Robinia pseudoacacia (Derr and

Evert, 1967), Populus tremuloides (Davis and Evert, 1968), the first sieve-elements arose from the undifferentiated cells that overwintered on the outer margin of the cambial zone. In other trees viz., Pseudotsuga taxifolia (Grillos and Smith, 1959) Tilia americana (Evert, 1962), Quercus alba (Anderson and Evert, 1965), Pinus strobus (Alfieri & Evert, 1968), Ulmus americana (Tucker, 1968) and Vitis riparia (Davis and Evert, 1970) the first functional sieve-elements were represented by the cells that overwintered in a matured state and were reactivated in spring.

According to Tucker and Evert (1969) sieve-element differentiation occured year round in Acer negundo.

M E T H O D O L O G Y

Materials :

The following species will be selected for the study of secondary phloem viz., *Aegle marmelos* Correa — Rutaceae; *Feronia limonia* Swingle — Rutaceae; *Tamarindus indica* Linn. — Legumiosae and *Zizyphus mauritiana* Lamk. — Rhamnaceae.

Aegle marmelos Correa

A medium-sized deciduous tree, 10-15 m tall, with yellowish grey bark, leaves digitately trifoliate, alternate, leaflets oblong, dentate. Flowers in axillary or terminal panicles, bisexual pentamerous, petals gland dotted. Fruit globose, rind woody, greenish or ashy grey, smooth, seeds many, embedded in orange coloured aromatic pulp, testa mucilaginous.

Leaf fall	Late March - April
Flowering	Late April - May
Fruiting	March - June
Local name	Bail (Husain, 1970).

Distribution :

Distributed throughout India and Pakistan, common in the Dehradun and Saharanpur forests and in other parts o the area. Wild or cultivated throughout the greater part of India, ascending

to 4,000 ft. on the outer Himalayas. Species 2 in Tropical Asia and one in Tropical West Africa.

Uses :

Wood is hard lustrous, suitable for house building, construction, agricultural implements carving, oil and sugar mills, pestles, tool handles combs etc. The gum obtained from the bark, used as a good adhesive. A yellow dye obtained from the rind of the unripe fruit is used in calico printing. An essential oil, marmelle oil is obtained from the rind. Fruits are edible, pulp is nutritious. Pulp mixed with lime forms a tenacious cement, used in construction of wells. The pulp has the detergent qualities and so it is used for washing clothes, also used as a varnish. Fruit has also the medicinal use for proper digestion.

Feronia limonia Swingle

A medium sized deciduous tree bearing thorny branches, bark grey to ashy grey. Leaves aromatic, imparipinnate, leaflets subsessile. Flowers polygamous, in panicles, male and female flowers usually on the same panicle. Fruit large, globose berry with woody pericarp, greenish white, rough, seeds numerous, embedded in an aromatic edible pulp.

Leaf fall	April
Flowering	Feb. - May
Fruiting	Jan-Feb. Fruit ripens in Oct. remains for a long time.
Local name	Kaith or Kaitha (Husain, 1970).

Distribution :

Throughout India and Pakistan. Siwalik range, and forests at the base of the Himalayas in Rohil Khand and North Oudh. Cultivated in many parts of India and occasionally found wild, also in Java. A single species.

Uses :

The wood is hard, durable and heavy, used for house building, naves of wheels, oil mills, agricultural implements ornamental carving.

The gum obtained from the bark is used as a substitute for the gum arabic for making water colours, dyes and varnishes.

The fruit is edible, nutritious, woody shell is used for fancy articles.

Tamarindus indica Linn.

A large evergreen tree with spreading crown, bark grey, rough, longitudinally and transversely fissured. Leaves paripinnate, leaflets coriaceous, oblong. Flowers in racemes, petals 3, subequal, yellow with red streaks. Pod thick, fleshy, pendulous, irregularly constricted between the seeds.

Leaf fall	Late Feb.-Sept.
Flowering	June - Sept.
Fruiting	Aug.-Sept.
Local name	Imli (Husain, 1970).

Distribution :

Throughout India and Pakistan planted for its fruit, shade also as an ornamental tree in gardens. Indigenous tree. A single species, planted everywhere in the tropics and probably a native of Tropical Africa.

Uses :

Wood is extremely hard, heavy, resistant to insect attacks, used for mallets, rice powders, oil and sugar mills mortars and pestles, agricultural implements, side planks of boats, cart wheels shafts, axles and naves, well construction, fuel etc. Charcoal prepared from this wood is used for gun-powder. Wood ash is used for tanning, dehairing goat skins. Bark is also used as a tanning material. The leaves, flowers and pods are used as auxiliaries in dyeing.

Fruits contain Citric, Tartaric and Malic Acids. The seeds too are medicinal. The acid flesh of the pod is used for cleaning metal vessels, its infusion with sea water is useful for cleaning silverware. Seeds are rich in the pectin jellose which is used

in jam and for painting images and idols. A cement is madeby mixing finely powdered seeds, with a glue it is one of the strongest wood cements, also used in sizing country-made blankets.

Zizyphus mauritiana Lamk.

A medium-sized deciduous heavily armed tree with stipular spines in pairs. Bark irregularly cracked, blackish brown, branches drooping. Leaves ovate- elliptic minutely denticulate glabrous above, rusty or grey beneath. Flowers in short axillary cymes, creamy white or yellowish green, strong smelling, Fruit globose or ovoid, berry red or orange when ripe, seeds 2 in each, stony and tubercled.

Flowering — Late Aug.-early Nov.

Fruiting — Jan.-Feb.

Local name — Pavendi Ber (Husain, 1970).

Distribution :

Throughout India and Pakistan, species about 40, found in Tropical Asia and America and in the temperate regions of both hemispheres. Common and gregarious along the base of the Saharanpur Siwaliks, also in Dehradun and in other parts of the area, frequently occurring as a mere shrub. Found throughout India and in Ceylon , wild and cultivated also in Tropical Africa, the Malay Archipelago, China and Australia.

Uses :

Wood is hard, tough and durable, takes a good polish, used for saddle, agricultural implements, well construction, oil mills, tool handles, sandals, golf clubs, gun stocks, shafts and axles of carts, naves, Persian wheels, fire wood etc. Bark contains 4-9 % of tannin and is used as a tanning material.

Fruit is edible and used for making refreshing beverages and also for dyeing silk.

Selection of the trees :

For each species about 50-60 fully grown, adult normal trees of comparable age and vigour will be selected. The selected trees will be numbered and labelled. Trees with abnormal growth due to shady or poor-soil conditions will be avoided since they are likely to show slow growth and other physiological disorders.

Collection :

The bark samples of one inch sq. size will be collected from the main trunks of the trees at chest height using a chisel and hammer. From each tree 4 such samples will be collected from opposite directions covering east, west, north and southern sides. The collections will be made once in fifteen days. In each turn the samples will be taken from three different trees. All collections will be made in the morning hours and will be fixed on the spot.

Fixing and preservation :

The samples will be fixed both in F.A.A.[1] and Craff III[2]. After reaching the laboratory they will be aspirated for the free access of the fixing fluid into the tissue systems. They will be remained in the fixative for 3-5 days and then will be transferred to 70% ethyl alcohol for preservation.

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1 & 2 — See APPENDIX.

Section-cutting :

The fixed samples will be washed in running water throughly. Sectioning will be done in a sliding micortome at a thickness of 10 to 12μ in transverse, tangential longitudianal and radial longitudinal planes.

Staining and dehydration :

The sections will be stained with a number of stains alone and in combination depending on the purpose of study.

To study the gross structure of bark the following combinations of stains will be used :

(i) Heidenhain's Iron Hematoxylin and Bismark Brown
(ii) " " " " Safranin
(iii) Safranin and Fast Green
(iv) Foster's Tannic acid-Ferric chloride
(v) Flemming's Triple stain.

For special purposes such as the detection of callose and P-protein some special stains will be used :

(i) Lacmoid in combination with Tannic acid and Ferric chloride (Cheadle, Esau and Gifford, 1953) for the dectection of callose
(ii) Ponceau S or Nigrosin for the detection of P-protein.

For macerated elements Astra Blue will be used for the sieve-tube elements and Potassium permanganate ($KMnO_4$) for the fibres.

In all the cases dehydration (to remove water from the material) will be done in ethyl-alcohol series. The schedule for staining with different stains is given below :

Heidenhain's Iron Hematoxylin and Bismark Brown :

Sections in 50% alcohol	canada balsam & cover slip.
↓	↑
30% alcohol	xylene III
↓	↑
Water	xylene II
↓	↑
Iron alum[3] 5 min.	xylene I
↓	↑
3 washings with D.W.	carbol-xylene
↓	↑
Heidenhain's Hematoxylin[4], differentiate under microscope or over stain	absolute alcohol
↓	↑
remove excess of stain by Iron alum 1-2 min.	90% alcohol
↓	↑
3 washings with water	70% alcohol
↓	↑
30% alcohol ——————→	50% alcohol
	↑
	wash in 50% alcohol
	↑
	Bismark Brown[5], 1-12 hrs.

NOTE :

In the above iron hematoxylin stains the cytoplasm as grey and middle lamella as black while bismark brown stains the lignified walls as brown.

3,4 & 5 - See APPENDIX.

Heidenhain's Iron Hematoxylin and Safranin[6] :

The schedule for this stain is the same as the above one except that the Bismark Brown is replaced by Sfranin (after 50% ethyl alcohol) for 1-12 hours.

Safranin and Fast Green[7]:

It is a general stain most commonly employed. In this case first the sections will be stained in aquous safranin (1-12 hr.) and then they will be dehydrated. After 95% alcohol sections will be stained in Fast Green in the clove oil medium (5-3 sec.). The excess of green will be removed in clove oil. After clearing in xylene mounting will be done in canada balsam.

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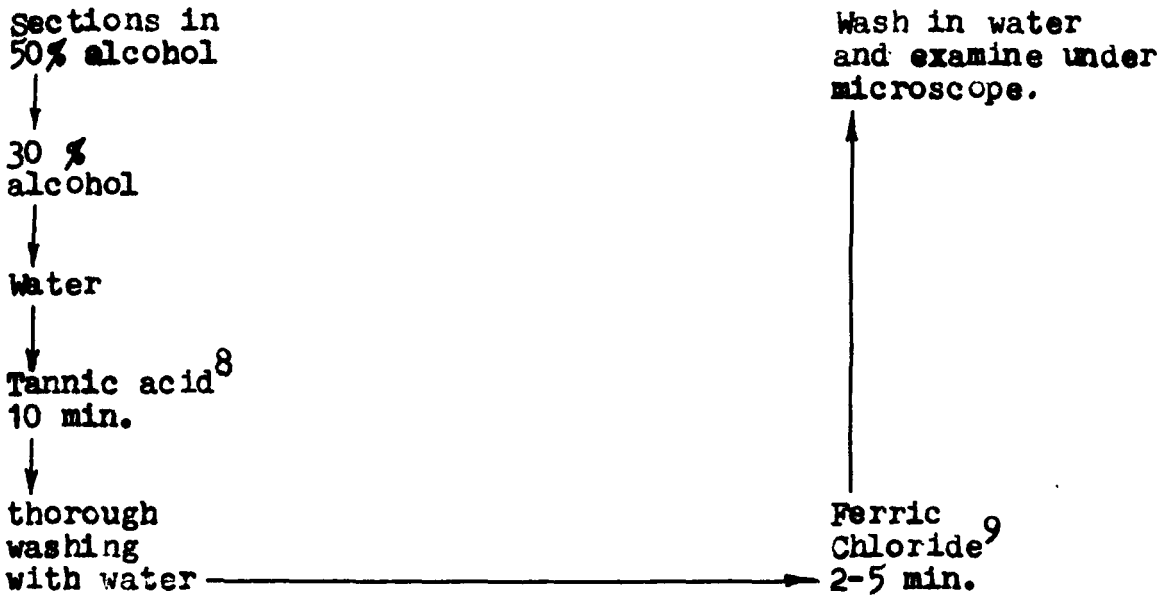
6 & 7 - See APPENDIX.

Tannic acid - Ferric Chloride Stain (Foster) :



NOTE :

It stains all the primary walls as brownish black.

8 & 9 - See APPENDIX.

Flemming's Triple Stain :
(Safranin, Crystal voilet and Orange G)

Safranin
4-24 hr.
↓
water, 3
changes
↓
Crystal voilet[10]
10 min. to 1 hr.
↓
water, 3 changes
↓
dip into 50%
alcohol
↓
rinse with
95% alcohol
(pipette)
↓
dip into
95% alcohol
↓
2-3 changes
anhydrous alcohol → flood sections with Orange G[11] → drain off Orange G
↑
rinse with
used clove oil
(pipette)
↑
flood with
new clove oil
↑
rinse with
clove oil-xylene
(pipette)
↑
differentiate
under microscope
↑
xylene
2 changes
↑
canada balsam
and cover slip.

NOTE :

Crystal voilet stains cellulose walls as voilet, orange G stains cytoplasm as orange and safranin stains the lignified walls as red.

10 & 11 - See APPENDIX.

Tannic acid-Ferric chloride-Lacmoid stain :

Sections in 50% alcohol
↓
30% alcohol
↓
water
↓
Tannic acid
5-10 min.
↓
3 washings
with D.W.
↓
Ferric chloride
5 min.
↓
3 washings with
D.W.
↓
Sodium hydrogen
carbonate[12] in 35% alc.
30 min.
↓
Lacmoid[13]
12-18 hr. →

Canada balsam
& cover slip.
↑
xylene III
↑
xylene II
↑
xylene I
↑
carbol-
xylene
↑
absolute
alcohol
↑
90%
alcohol
↑ 2-3 min.
70%
alcohol
↑ 2-3 min.
Sodium hydrogen
carbonate in 50% alc.
few sec.

NOTE :

Tannic acid-Ferric chloride stains the cytoplasm as brown while the Lacmoid stains the callose and lignified cells as blue.

12 & 13 - See APPENDIX.

Ponceau S (Single stain):

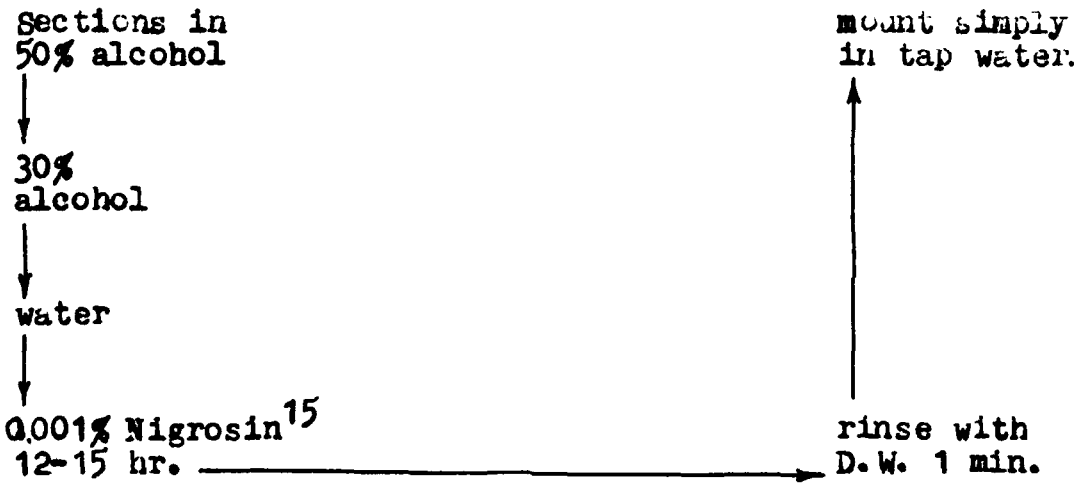
Sections in
50% alcohol
↓
30%
alcohol
↓
Water
↓
Ponceau S[14]
5 min. ——→ 2 changes in
aquous 5% acetic acid
(5 min. each change)
↑
Temporary mounts may be made in tap water or Glycerin (5%) or may be dehydrated & mounted in canada balsam as given in other charts.

NOTE :

It stains the P-protein strands as pink.

14 - See APPENDIX.

Nigrosin (Single stain):



NOTE :

It stains the P-protein strands as dark blue.

15 - See APPENDIX.

Maceration Technique :

A simple maceration technique has been evolved recently (Ghouse et al., 1974) to obtain the sieve-elements and fibres in macerated conditions to study their morphological and histochemical variations in the different stages of their functional period and in the different seasons.

2 cm. square blocks of the bark containing the cambium and the conducting phloem were removed from the main trunks of woody plants, using a chisel and hammer, fixed in F.A.A. for five days, and then thin tangential slices (0.5 mm thickness) were made. The above slices were then treated with 5% NaOH solution for 3-5 days at 45-50°C, to soften the tissues. After 72 hrs, they were transferred to fresh NaOH solution of the same concentration. Periodical checking was made to know the condition of the treated slices. The treatment was continued till the cells of the slices became sufficiently loose to allow the separation of the individual elements on a slide when a slight pressure is applied over the coverslip after mounting.

When the desired stage was reached, the slices were washed and stained in 1% aqueous solution of either astra blue or lacmoid for 12-24 hours (both for sieve-elements), depending on the season of collection. The latter was preferred in winter to make clear the closure of the sieve pores by callose.

Astra Blue (for sieve-elements):

1. Macerated sieve-elements were washed throughly with tap water to remove NaOH.
2. Material was dipped in 1-2% Astra Blue prepared in D. W. for overnight.
3. Mounted in 5% glycerin or dehydrated and mounted in canada balsam.
4. Stains the sieve-elements as blue.

$KMnO_4$ - Potassium permanganate (for fibres):

1. Macerated material was washed with water throughly.
2. Dipped into 2-5% $KMnO_4$ solution prepared in D. W. for overnight.
3. Mounted in 5% glycerin or dehydrated and mounted in canada balsam.
4. Stains the fibres as dark brown.

Plan of study :

The study of the problem 'The formation and functioning of phloem', will include the following aspects :

1. Study of bark structure :

The gross structure of the bark of the selected species will be studied in transection to find out composition and the spatial relationships of the different components of secondary phloem.

2. Study of morphological characteristics of the principal components :

The morphological features of the sieve-elements, fibres and sclereids will be studied in macerated material.

3. Determination of the duration of cambial activity :

The duration of cambial activity will be determined out of the fortnightly collections for three calendar years. For this purpose attempts will be made to recognise the active form of cambium from its dormant phase using some already recognised useful features of the cambial cells, such as the number of layers of cells in the cambial zone, thickness and the beaded nature of the radial walls of the fusiform initials, size of the nucleus, cell content and the cell inclusion like starch in the ray initials.

4. Seasonal incre-ment in the amount of phloem :

This will be determined in the fortnightly collections by using the following two criteria :

a) Nature of sieve-element in a given period i.e. how long

they remain intact without undergoing deformation.

b) Nature of contents including the slime.

c) Amount of callose depositon.

5. Determination of the functional duration of the sieve-elements.

6. Interpretation of the results in the light of the work done in the past.

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*Not seen in original.

A P P E N D I X

[1]F.A.A.

This is a mixture of Formalin, Glacial acetic acid and Ethyl alcohol (Formalin-aceto-alcohol) in the following proportion:

Ethyl alcohol (50%).....90cc
Glacial Acetic acid..... 5cc
Formalin 5cc

[2]Craff III

It is also a mixture of chromic acid, acetic acid, formaldehyde and water in the following proportion :

1% Chromic acid 30cc
10% Acetic acid (not glacial)..... 20cc
Formaldehyde 37-40% (aqueous)..... 10cc
Water..... 40cc

[3]Iron alum (Ferric Ammonium Sulphate)

Used in this stain as a mordant or a substance to fix the dye 0.4% solution of iron alum is made in distilled water.

[4]Hematoxylin

A homologue of brasilin. The stain itself has little or no affin-ity for tissues unless iron (always in the ferric form) or aluminium is present in the latter. The solution of the dye is

0.5% solution in distilled water. Stain is dissolved in boiling water (do not boil the solution) cool it.

[5]Bismark Brown

Basic; azo group. Solubility : 1.36% in water; 1.08% in alcohol. A 1% solution in 70% alcohol is better. Rarely it overstains and is quite permanent. It stains the mucin and cellulose walls as brown. Solution of the dye should never be heated. It works poorly on materials fixed in the reagents containing chromic acid.

[6]Safranin

Basic azin group. Solubility 5.45% in water; 3.41% in alcohol 0.5 to 1% solution of the stain made in 50% ethyl alcohol. It stains cutin, chromatin, lignin and in some cases chloroplasts. In using it, before proceeding the differentiation one should wash out excess of stain with water.

[7]Fast Green

Acid. Diamino-triphenyl methane group. Solubility 16.04% in water; 0.33% in alcohol. 0.5 to 1% solution in 95% ethyl alcohol. Used for differentiating safranin. It stains all tissues.

[8]Tannic acid and [9]Ferric Chloride

Tannic acid acts as a mordant in this combination. 1% aquous solution is sufficient. Ferric Chloride solution is 3% in water.

Both stain all the primary walls as brownish black.

[10]Crystal voilet (or gentian voilet)

Basic; triamino - triphenyl methane group. Solubility : 1.68% in water; 13.87% in alcohol. A 1% solution in distilled water is sufficient. The dye washes out in the dehydrating alcohols therefore it is dissolved in clove oil. It frequently overstains the cytoplasm. It stains the cytoplasm and nucleus.

[11]Orange G

Acid; azo series. Solubility : 10.86% in water; 0.22% in alcohol. It is a useful cytoplasmic stain. Medium in which the dye is used varies according to the specific technique. 1% solutions of the dye is used.

[12]Sodium hydrogen carbonate

Used as a mordant for Lacmoid. 1 % solution in 35% ethyl alcohol is sufficient.

[13]Lacmoid (or Resorcin Blue)

Basic; oxazin series. Used as a microchemical reagent for callose.

[14]Ponceau S

Special stain for P-protein. 0.2% solution of the dye in 3% aquous trichloroacetic acid is used. It stains the strands as pink.

[15] **Nigrosin** (or Indulin black)

Basic; Stains the P-protein. 0.001% of the dye in 2% acetic acid is used. It stains the P-protein as dark blue.
